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THE AMERICAN REVIEW OF TUBERCULOSIS

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ALLEN K. KRAUSE, Baltimore, Md.

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(16) DeWitt, L. M.: Action of mercurochrome-220 and of mercurophen. A preliminary report of effects on the human tubercle bacillus and on experimental tuberculosis in guinea pigs, J. Am. M. Assoc., 1920, lxxv, 1422–3.

(17) Corper, H. J., and Robin, H. A.: The pulmonary aspiration of particulate matter, Am. Rev. Tuberc., 1922, vi, 813, and 1923, viii, 386.

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TUBERCULOSIS IN THE TROPICS

A Review of Findings in 492 Autopsies Performed in São Paulo, Brazil[1]

ROBERT A. LAMBERT AND BONIFACIO DE CASTRO FILHO[2]

The manifestations of tuberculosis in the tropics have long been subjects of special interest among students of this disease, with the result that many data relating especially to the incidence and clinical and anatomical characteristics of the infection among the inhabitants of warm countries have been accumulated. These data have been well summarized by Bushnell (1) in his *Epidemiology of Tuberculosis*, along with a broad discussion of the subject. However, among the numerous reports brought together in this treatise, one finds only two or three relating to tuberculosis in South America, the continent which, next to Africa, contains the largest territory having a tropical or subtropical climate. Furthermore, relatively few of the investigations are based on autopsy findings. It has therefore seemed to us worthwhile to record the findings as respects tuberculosis in a series of some four hundred autopsies made in the past four years in São Paulo, the capital of one of the largest and most populous states of Southern Brazil.

It should be stated at the outset that the climate of this portion of Brazil, except for the narrow coastal plain, is not truly tropical. The State of São Paulo is bisected by the Tropic of Capricorn, its territory being thus shared by the South temperate and torrid zones. Furthermore, the greater part of its area has an altitude of 1000 to 2000 feet, which tempers considerably the tropic heat. Marked fluctuations in temperature, amounting to as much as 40°F., with rarely extremes of 40° and 100°F., and a pronounced humidity throughout a large part of the year, are noteworthy features of the climate, particularly in the eastern portion of the State. Frost is rarely seen except in the higher alti-

[1] A large majority of these autopsies were performed by Dr. João Montenegro, assistant in pathology and pathologist to the Santa Casa, and the authors take pleasure in acknowledging herewith their obligation to him for the careful records on which this study is largely based. Acknowledgment is also made to Dr. Oskar Klotz, Professor of Pathological Anatomy during the first half of the period covered by this study.

[2] Institute of Pathological Anatomy, Faculty of Medicine, São Paulo, Brazil.

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tudes; so that, in general, the conditions of living approximate closely those of tropical countries.

MATERIAL INVESTIGATED

This study is based on 402 autopsies on unclaimed bodies obtained from the Santa Casa de Misericordia, the large hospital of one thousand beds[3] of Sào Paulo. Of the total, 345 were males and 57 females. As the following table shows a large majority were adults.

AGE IN YEARS	NUMBER OF CASES
1–9	14
10–19	25
20–29	94
30–39	86
40–49	66
50–59	63
60–69	41
70–79	12
Not recorded	1
	402

The cases were, in general, unselected; that is, the cadavers were delivered to the department of pathology on certain days of the week for class demonstrations upon a standing requisition from the medical school. In only a small number, less than 10 per cent of the total, were the autopsies performed by reason of any special interest in the cases on the part of clinicians or others. It may, therefore, be assumed that the prevalent diseases of the country would be fairly well represented in such a collection of autopsies. It should be noted, however, that the mortality in the tuberculosis wards, which contain 70 beds, occupied almost exclusively by advanced cases, is relatively high; thus giving perhaps among the deaths in the institution an unduly large percentage of lethal tuberculosis.[4] It may also be suggested that among the tuberculous a large number of "drifters" may be expected, resulting in a larger proportion of unclaimed cadavers. This is possibly true, although on account of the facts (1) that in Sào Paulo, as elsewhere, a large proportion of those admitted to the hospital belong to the class of indigent poor

[3] The normal capacity of the hospital is 500 to 600, but for more than ten years the institution has been compelled to provide for a daily average of around 1000 patients, the number reaching 1100 to 1200 at times.

[4] More than half.

who come from the country districts and do not keep in touch with their families, and (2) that the law requires that the bodies of deceased persons be claimed within twenty-four hours, it is not surprising that unclaimed bodies are more numerous and more representative than in the general hospitals of most of our large North American cities. During the first three months of 1925, a representative period, approximately 50 per cent of the cadavers of patients dying in the Santa Casa were unclaimed, and were therefore available for autopsy or anatomical study.

FINDINGS

Although the autopsies were performed with no special interest in tuberculosis, in practically every case the examination was complete and included the brain. All organs were systematically described. Interesting and doubtful lesions were, as a rule, investigated histologically, and in those cases in which the tissues were fairly well preserved sections were made of the various viscera without regard to the gross findings. It is, therefore, reasonably certain that no case of lethal tuberculosis escaped detection, and that probably only an occasional case of subsidiary tuberculosis was overlooked. On the other hand, it is probable that a fair number of obsolete or healed lesions, such as an isolated calcified node or a small apical scar, were not recorded.

In summarizing our findings we have classified the cases as follows:

1. *Lethal* tuberculosis, that is, those in which tuberculosis was the cause of death or one of two stated causes.
2. *Subsidiary*, in which an apparently active infection was present, but not sufficiently extensive to have contributed materially to the death of the subject.
3. *Healed*, in which only calcified or cicatrized lesions were demonstrably of unquestionable tuberculous origin.
4. *Doubtful*, in which scars or fibrous adhesions of possible tuberculous origin were found. These cases were principally those showing more or less extensive fibrous pleural adhesions, in which the description did not indicate any other evidence of tuberculosis. Pericardial and localized peritoneal adhesions were not included.
5. *Negative*, in which no lesion suggestive of tuberculosis infection was recorded.

Since the age of the patient is of special interest in the interpretation of the findings, we have combined in the following table the numbers and percentages of the five groups of cases in relation to age periods.

AGE	TOTAL NUMBER	TUBERCULOSIS				
		Lethal	Subsidiary	Healed	Doubtful	Negative
1–9	14	2			1	11
10–19	25	6	5		8	6
20–29	94	29	10	3	27	25
30–39	86	21	14	6	21	24
40–49	66	15	12	4	22	13
50–59	63	5	13	3	32	10
60–69	41	6	5	2	15	13
70–	12		3		6	3
	401	84	62	18	132	105

It should be emphasized that the figures for *healed* refer to cases in which *only* healed lesions were found and do not include the total incidence of such lesions, since in many of the cases of lethal and subsidiary tuberculosis anatomical evidence of healing was found. In the lung alone healed lesions were specifically mentioned in the anatomical diagnosis of 31 cases.

The *doubtful* cases unquestionably include many lesions of nontuberculous origin. As stated above, these cases were mostly instances of old organized pleural adhesions. The influenza epidemic of 1918–1919, with its complicating pneumonias and pleurisies, may account for many of these adhesions, as well as for some of the instances of pulmonary fibrosis. During the past year we have seen several examples of more or less diffuse fibrosis of the lung, two of which were so extensive as to simulate fibroid phthisis, but which after careful study were interpreted as the end-result of an organizing influenzal pneumonia. Granting that 50 per cent of these *doubtful* lesions were nontuberculous, it is seen that the incidence of healed infection approaches that obtained in some of the similar studies among the tuberculized people of the United States and Europe (Opie (2), Burkhardt (3), Wason (4) and others). Such an "approximation" regarding the nature of such doubtful lesions may be questioned. It is admitted that a careful weighing of the evidence in each case would give a much more accurate estimate. But in the absence of detailed data, it has seemed justifiable at least to suggest this approximation, in which, we believe, the error is not large.

The percentages, with relation to age, are shown in the following table:

AGE	DEFINITE TUBERCULOSIS	DEFINITE TUBERCULOSIS PLUS 50% OF DOUBTFUL CASES
1–9	14	18
10–19	44	60
20–29	44	59
30–39	47	60
40–49	46	63.5
50–59	34	59
60–69	31	50
70–	25	50

It is seen that the incidence for the 10–19 year period was rather higher than might have been expected, being practically the same as that of the higher decades, but this is explained in part by the fact that the total number in this group was relatively small, and that most of these belonged to the postadolescent period. The smaller amount of both lethal and subsidiary tuberculosis in the higher decades (50–70) is noteworthy.

RACE

Of the 402 cases, 263 were recorded as "white," 110 "negro," 24 "brown," that is, dark mulatto,[5] and 5 "yellow" (Chinese and Japanese).

The rather striking difference in the findings in the two chief racial groups, white and negro, is well shown in the following table:

	WHITE	NEGRO
	per cent	*per cent*
Lethal	15.5	27.3
Subsidiary	16.0	17.3
Healed	5.9	0.9
Doubtful	36.0	31.8

The dark mulattoes showed even a higher percentage of lethal tuberculosis (8 out of 24 cases) than the negroes, with only a single case in which the lesions were entirely healed. Of 31 instances of *healed* lesions in the lungs, 27 were in whites and only 4 in negroes, an incidence nearly seven times as great in the whites, whereas *lethal* tuberculosis was nearly twice as common among the colored. (The figures for healed lesions, however, were too small to permit a fair comparison. Very likely the ratio 27:4 is an exaggeration of the difference.)

[5] Undoubtedly the "whites" in this series include a certain number of light mulattoes.

ANATOMICAL FEATURES

Pulmonary tuberculosis, as might be expected, dominated the picture in a large majority of the cases. Of the 84 fatal cases all showed lesions in the lungs, approximately 75 per cent of these being more or less extensive caseating processes, and the remainder miliary. The type of pulmonary lesion varied from the typical chronic phthisis, with evidence of attempts at repair and localization, to the rapidly progressive caseous pneumonia. It was our impression, however, that the percentage of cases of the pneumonic type was larger than we had been accustomed to see in similar series of autopsies in the United States. It was noteworthy that a majority of the cases of acute generalized tuberculosis were found among colored males between the ages of 18 and 30.

Lesions in the larynx or trachea were found in 11 cases, all instances of lethal pulmonary tuberculosis.[6]

The intestines were involved in 40 cases, all instances of lethal tuberculosis. In 30 of these the infection was secondary to a more or less extensive pulmonary lesion, the others being instances of tuberculous peritonitis or general miliary tuberculosis. Lesions in the appendix were found five times.

Involvement of the meninges was present in 9 cases, in all of which the infection was generalized.

Involvement of the peritoneum was found in 16 cases, in 5 of which the peritonitis was the chief focus of the infection and the stated cause of death, the other cases being instances of generalized tuberculosis. Tuberculous pericarditis was noted 3 times, while no lesions of the myocardium or endocardium were observed.

Tubercles were recorded in the liver 37 times, spleen 37, kidney 25, prostate 1, and epididymis 1.

The records for the lymph nodes show the following: The thoracic nodes were involved in 105 cases, the abdominal in 36, and the cervical in only 6. Of the 105 instances of thoracic involvement, 41 were nonlethal infections, while of the 36 abdominal (chiefly mesenteric) nodes affected 34 were in cases of lethal tuberculosis. Of the 6 cervical, 5 were lethal. In other words, subsidiary or healed tuberculosis of the cervical and mesenteric nodes, a not uncommon finding in our North American autopsies, seems to be a comparatively rare phenomenon in Brazil, if

[6] Since this study was made we have encountered a case of extensive tuberculous ulceration of larynx and pharynx with only slight pulmonary involvement.

our material be accepted as representative. (Metcalf (5) says; "Tuberculous infection of these (mesenteric) nodes is common, caseous or calcified nodes being frequent at autopsy.") The reasons for this significant difference will be discussed in a subsequent paragraph.

There is little to record respecting bone tuberculosis, except its striking rarity. Only a single instance of caseous involvement was found, this a case of typical Pott's disease, in which death resulted from a generalization of the infection. It is possible, of course, that an occasional case of early or slight bone involvement may have escaped notice, but the fact remains that among more than four hundred autopsies only one case of manifest bone tuberculosis, and none of the joints, tendons or skin, was seen.

DISCUSSION

It is recognized that the anatomical manifestations of tuberculosis, as seen in the fixed population of any country, vary according to the degree of immunity, that is, the "tuberculization" of the people. Or, stated conversely, the extent of tuberculization of a people may be judged by the reactions in their tissues which the tubercle bacillus is capable of inducing. On this basis, Bushnell divides races and people into three groups: (1) the "more or less well immunized," (2) the "imperfectly immunized," and (3) those having "little or no immunity." The first is characterized by the predominance of chronic pulmonary lesions with a tendency to fibrosis, localization, the walling off of cavities, etc. In fatal cases, the infection is often found to have spread only to the upper respiratory tract or, by the same route, to the intestines. Dissemination by the blood-stream does not necessarily result in a generalized tuberculosis, since not infrequently healed tubercles are found in the viscera, spleen, liver, kidney. Lymph-node involvement is common, but not extensive and progressive. In a large proportion of the cases the process becomes arrested, hygienic conditions being favorable.

According to Bushnell, the chief characteristic of the "imperfectly immunized" is the tendency to extensive and progressive involvement of the lymphatic system, with a tendency to secondary spread to various organs, notably the lungs, kidneys, intestines and genital glands. Involvement of the skin, bones and eyes is regarded as another feature of this imperfect immunization.

The third class, nonimmunes, presents generally the type of tubercu-

losis seen in the young child, that is, a rapidly generalizing process with no tendency to localization or healing. The reaction, particularly in the case of pulmonary infection, is exudative rather than proliferative, caseous pneumonias, for example, being quite common.

It is this last type of tuberculosis which is popularly associated with the tropics, owing no doubt to a number of striking examples of the devastating effect of the infection on certain primitive tropical peoples, suddenly brought in contact with "civilization;" the depopulation of many of the South Sea Islands, the terrible mortality among Mozambique negroes brought to India, the similar fate of Polynesians taken to Peru, etc. But it is very probable, as Bushnell and others suggest, that the climate and race *per se* have little or nothing to do with such manifestations. The same phenomena have been observed in very different races living in cold climates, as for instance, the Indians of Terra del Fuego, who in the process of being "Christianized" by the Chileans were almost annihilated by the tuberculosis which the well-meaning priests introduced.

Reviewing the findings in our series in the light of the above classification, certain deductions are at once apparent.

In the first place, as was to have been expected from the history of Brazil, peopled largely by Europeans, with several centuries of close contact with the mother countries, the character of the tuberculous lesions found indicates, particularly among the whites, a fairly high degree of immunity comparable to that seen in Europe and North America. It is noteworthy that, as in the United States, the negro, in spite of an association of several generations, at least, with the whites, shows very clearly a lower degree of immunization, as evidenced by the rarity of healed lesions and the frequency of generalized and pneumonic types of infection. The much higher incidence of lethal tuberculosis among the negroes in our series may be partly accounted for by their lower scale of living, greater ignorance of proper hygiene, etc.

In respect to these racial differences, it may be noted, in passing, that we did not observe the tendency to involvement of the heart, such as Clark (6) recorded in a series of autopsies on 432 West Indian negroes dying in Panama. He observed 62 instances of tuberculous pericarditis, 15 of myocarditis, and 6 of endocarditis. Or our 3 cases of tuberculosis of the pericardium, however, it is possibly significant that all were in negroes, and that peritoneal and meningeal involvement also occurred

more frequently in the colored, another indication, according to Bushnell, of a lesser degree of immunity.

The explanation of the relatively small number of instances of subsidiary or healed tuberculosis of the cervical and mesenteric lymph nodes in our series (one of the former, two of the latter) is to be ascribed, in our opinion, to the universal custom of the country of boiling all milk before using. The above statement is based on the assumption, well supported we believe, that primary infection of those lymph nodes is generally of bovine origin. It should be pointed out that tuberculosis of cattle and hogs, as slaughter-house records show, is very prevalent in Brazil, particularly in some of the dairy districts.[7]

The low incidence of primary bone tuberculosis, also commonly assumed to be a bovine infection, may be explained in the same way. However, accepting Bushnell's statement that secondary bone involvement is frequently seen in the imperfectly tuberculized, we should have expected to see instances of such extension, at least among the negroes.

It may be added that our observations, in respect to the low incidence of bone and lymph-node tuberculosis among autopsies, coincide with those of several local clinicians of large experience. One of these, a dermatologist, also emphasized the rarity in local clinics of cutaneous tuberculosis, as compared with Europe. The possibility of the influence of sunlight, should, of course, be borne in mind in the discussion of these differences.

SUMMARY

A systematic study of the anatomical findings in 402 autopsies in Sào Paulo, Brazil, revealed the following facts:

1. Lethal tuberculosis was found in 83 cases, or 20.6 per cent; subsidiary tuberculosis in 15.4 per cent; healed lesions only, in 4.5 per cent; and "doubtful" lesions only, chiefly instances of more or less extensive pleural adhesions, in 32.3 per cent.

Assuming that half the "doubtful" lesions were tuberculous in origin, the percentage of cases showing evidence of active or healed infection was

[7] It is of interest to note that Whitmore's observations in the Philippines (*Phil. Jour. Sci., 1909, iv, 453*) do not support this interpretation. He found that, whereas hospital and dispensary records in Manila indicated that "surgical tuberculosis" was as common there as in the United States and Europe, native cattle were said to be very rarely infected. Furthermore, milk and butter entered but slightly into the dietary of the people. These findings, he pointed out, spoke against the bovine origin of bone and lymph-node tuberculosis.

approximately 60 per cent. The percentage was practically the same for the second, third, fourth and fifth decades of life.

2. The percentage of lethal tuberculosis in the negroes was almost double that in the whites, 27.3 and 15.5, respectively, whereas there were almost four times as many whites as negroes in which undoubted healed lesions were demonstrable.

3. The anatomical character of the lesions, particularly among the whites, corresponds in general to that seen in the "tuberculized" peoples of Europe and North America, typical chronic pulmonary tuberculosis being the chief manifestation of the infection in a large majority of the cases.

4. Except as a part of a generalized infection, involvement of the cervical and mesenteric lymph nodes was rarely seen.

Among the groups of "subsidiary" and "healed" cases only one instance of cervical and two of mesenteric involvement were found.

Only one example of tuberculosis of the bones and none of the joints, skin or tendons was seen.

It is suggested that the custom of using only boiled milk may account for the apparent rarity of the bovine type of infection.

5. The high percentage of lethal tuberculosis in the series is probably considerably greater than would be found in a more truly representative group of cases, owing to the high mortality among the advanced cases admitted to the general hospital, from which the cadavers were obtained. We believe, however, that the findings serve to emphasize the fact, generally recognized by the hygienists of the country, that tuberculosis, as in many of the countries of the north temperate zone, constitutes one of the greatest scourges of this section of tropical America.

REFERENCES

(1) Bushnell, G. E.: A study in the epidemiology of tuberculosis with special reference to tuberculosis of the tropics and of the negro race, New York, 1922.

(2) Opie, E. L.: Focal pulmonary tuberculosis of children and adults, J. Exper. Med., 1917, xxv, 855.

(3) Burkhardt, A.: Ueber Häufigkeit und Ursache des menschlichen Tuberkulose, Ztschr. f. Hyg., 1906, liii, 139.

(4) Wason, I.: Tuberculous lesions in six hundred autopsies, Trans. Eighteenth Annual Meeting, Nat. Tuberc. Assn., Washington, 1922.

(5) Metcalf, W. R.: Tuberculosis of the lymphatic system, New York, 1919.

(6) Clark, H. C.: Pathological notes pertaining to tuberculosis in the Negro of the Panama Canal Zone, Amer. J. Trop. Dis. and Prevent. Med., 1915–1916, iii, 331.

A CASE OF BILATERAL SPONTANEOUS PNEUMOTHORAX

ROWLAND BRIGGS[1]

Spontaneous bilateral pneumothorax is a very rare condition, as judged by the paucity of published reports. The etiological factors are (1) trauma and (2) rupture of a cavity near the periphery of the lung. The origin of the cavity may be syphilitic, asthmatic, empyemic, tuberculous, or the result of a tumor such as cysticercus or one of malignant nature.

Altogether, there have been but 8 cases reported. Meyer in 1917 described a case of spontaneous bilateral pneumothorax, unusual inasmuch as no evidence of active tuberculosis could be demonstrated at autopsy. The etiological factor in this case was attributed to the exertion of coitus, as the condition recurred on four occasions, each time immediately following sexual intercourse. In this case aspiration of air occasioned but temporary relief. Marshak in 1918 reported a fatal case of double spontaneous pneumothorax, in a man aged fifty, which was a complication of pulmonary tuberculosis. Gray in 1919 described a case of bilateral spontaneous pneumothorax of tuberculous origin, which at first experienced some relief following the aspiration of air. This patient died shortly afterward, no autopsy being obtained.

In 1921 Bourne described a fatal case of bilateral pneumothorax, observed in an infant, which was found upon autopsy to be the result of an extensive miliary tuberculosis. Hawes in 1922 described a case of recurrent bilateral pneumothorax of tuberculous origin. This patient underwent numerous exacerbations during the period of nearly ten years that the author had him under observation, with ultimate recovery. Eberts in 1923 described a case of bilateral pneumothorax cured by negative tension. This case was primarily one of influenza, complicated by pneumonia, which was followed by a bilateral empyema, and in turn by a bilateral pneumothorax. Following the use of a negative-tension apparatus both cavities disappeared. Emerson and Beeler in 1923 describe a fatal case of spontaneous bilateral pneumothorax of asthmatic

[1] Mercy Hospital, Pittsburgh, Pennsylvania.

origin. This case was exceptional because of the absence of any evidences of tuberculosis.

Grashey states that spontaneous pneumothorax is not infrequently observed. The diagnosis is easy, except that sometimes a small flat

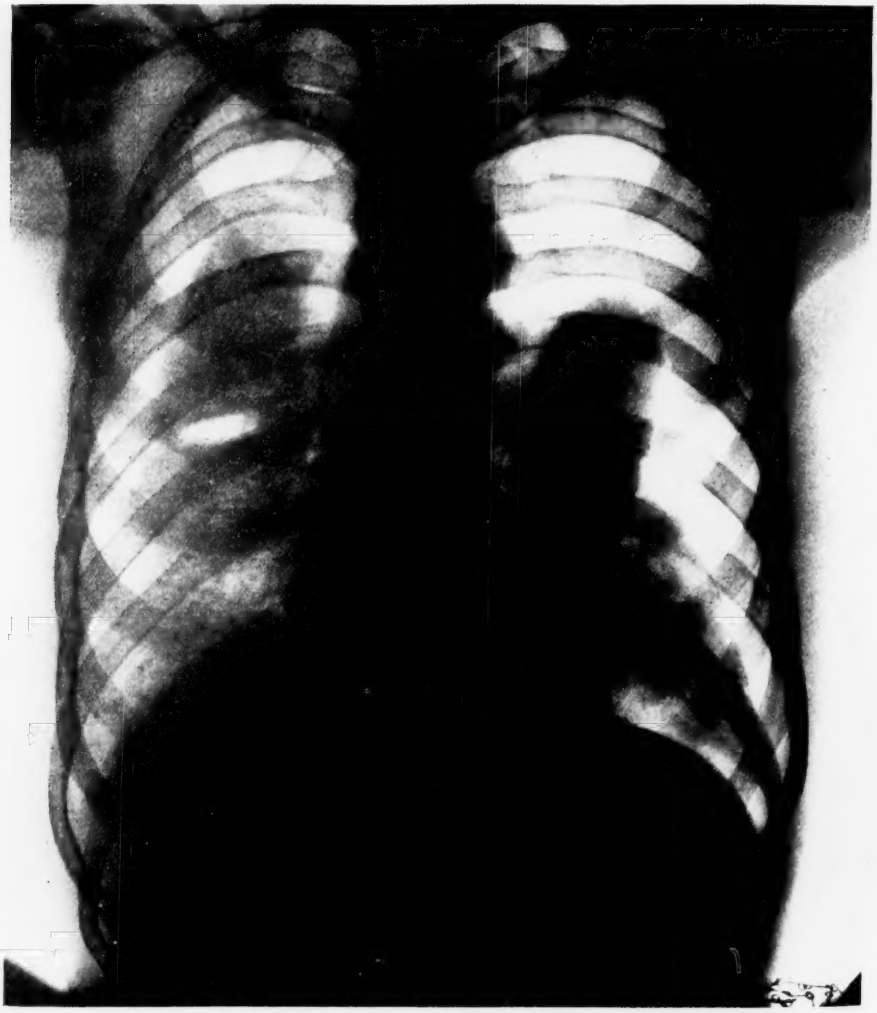


FIG. 1. BILATERAL PNEUMOTHORAX

pneumothorax, situated at the apex, will be overlooked, or an error of diagnosis made. The condition is nearly always due to tuberculosis and the prognosis in those cases is usually bad, this being particularly true when there is a mixed infection. Grashey describes two cases, one

a complete pneumothorax on one side and partial pneumothorax on the other. In the second case the patient had sudden pain on one side, and upon examination a complete unilateral pneumothorax was found; six months after the aspiration of air the patient was normal.

Because of the rarity of this condition it has seemed of value to report the following case, which was encountered on the medical service of the Mercy Hospital, Pittsburgh, during a routine X-ray examination of the gastrointestinal tract. Inasmuch as its presence had not been previously suspected, it was too late when discovered to institute effective therapeutic measures. Very illuminating X-ray studies however were made (fig. 1).

Case no. 19428. Age 50 Years

Preliminary note: For nearly twenty years before his admission to the medical service of the Mercy Hospital, Pittsburgh, the patient was employed in a steel mill in the capacity of "steel blower." His work consisted in directing, by mechanical means, compressed air upon molten metal, in an attempt to oxidize impurities, and he frequently made use of a preparation containing sulphur to aid in the oxidation. The atmosphere in which he worked was very dusty and frequently disagreeable, owing to the volatile products of combustion of the sulphur preparation.

Complaint: Shortness of breath and loss of appetite.

Family history: The patient's father was killed in an accident at the steel works. His mother is living and in fair health at the age of 75 years. Two brothers are living and well at ages 52 and 38. Five sisters are living and well at ages 56, 54, 47, 43 and 30. One brother died at 4 years of age of spinal meningitis. One maternal aunt died of carcinoma of the stomach when 72 years old. With this exception there was no family history of malignant tumor, tuberculosis, heart or kidney disease, arthritis, goitre, obesity, or nervous affections such as epilepsy, insanity, etc.

Personal history: Previous to the onset of his present illness the patient always considered himself in excellent physical condition. He had measles, mumps and whooping cough in childhood, and uncomplicated typhoid fever when 16 years old. Seven years ago he had what was called "congeston of the lungs with pleurisy." He had never had diphtheria, scarlet fever, rheumatic fever or malarial fever.

Present illness: The onset was in August, 1923, when shortness of breath was first noticed. This was attributed by the patient to the sulphur in the atmosphere in which he worked. At this time there was a diffuse pain on both sides of the chest, which seemed more marked on the right side. The onset was gradual, and the pain was not localized. This attack, the cause of

which was attributed by his physician to "heart trouble," lasted about a week, during which time he was compelled to go to bed. His physician prescribed digitalis, which seemed to relieve him, and at the end of the week the patient had recuperated sufficiently to resume his work. He continued at work until August, 1924, when he again noticed shortness of breath, this time becoming progressively worse. This second attack was accompanied by severe coughing spells, especially in the morning, and with an occasional vomiting spell. Since August, 1924, he had noticed rapid emaciation and he had lost 30 pounds in the four months preceding admission, but he never at any time coughed or vomited blood or passed blood by bowel.

Physical examination: The patient is a moderately well-developed man, lying quietly in bed. His facial expression is one of apprehension, yet he shows no evidence of respiratory distress, and appears to be comfortable. He is obviously emaciated, very little of the panniculus remaining, the skin is dry and of slightly yellowish tint, and the mucous membranes are pale. *Mouth:* All teeth are missing, the tongue is heavily coated, and the breath foul. *Neck:* There is a slight struma. *Thorax:* Owing to the patient's emaciation the supraclavicular and infraclavicular fossae are unusually deep. Respiratory movements are shallow, though not labored. The costal angle is approximately 90°. When asked to inhale deeply, the expansion of the patient's thorax is definitely limited on both sides, and no evidence of Litten's sign is visible. The scapulae are winged and unduly prominent, the spinous processes of the vertebrae are prominent, there is no scoliosis. Vocal fremitus is practically absent, except at the right base, where it is considerably decreased in intensity. The percussion note over the entire chest, both front and back, is definitely tympanitic, except at the right base where there is slight resonance. The breath-sounds are unusually distant, being scarcely audible anywhere except at the right base. Here a few moist râles are heard. *Heart:* The precordium shows, on inspection, nothing unusual except for the absence of *P.M.I.* Upon palpation the *P.M.I.* is felt in the sixth interspace, 7 cm. to the left of the midsternal line. The area of absolute cardiac dullness extends only 3 cm. to the left of the midsternal line in the sixth interspace. The heart-sounds are very distant, the rhythm is regular, and no murmurs are heard. *Pulse:* The radial pulses are synchronous and equal, of good volume, regular rhythm, and 84 per minute. The arterial walls are moderately sclerosed. The blood pressure is 125/80. *Abdomen:* The abdomen is of usual size and shape; no masses or dilated veins are visible. The walls are rather tense. There is an indefinitely localized area of tenderness in the right upper quadrant, and on light pressure there is felt a mass about the size of a small lemon, about one finger's breadth below the costal margin on the left, between the midclavicular and parasternal lines. The movements of this mass are synchronous with those of respiration. The liver is not

palpable. The kidneys are palpable but not abnormally mobile. The spleen is not enlarged.

Laboratory tests: Urine: Negative on two examinations. *Blood:* R.B.C. 4,800,000; W.B.C. 8,800; Hb. 85 per cent. *Gastric analysis:* Showed blood ++, in addition to an absence of free hydrochloric acid. *Stool examination:* Blood+; mucous +; parasites negative. *Sputum:* Negative for tubercle bacilli on three examinations. *X-ray studies:* Fluoroscopic examination of the chest presents a unique appearance. There is a large, dense, sharply circumscribed, kidney-shaped shadow of atelectatic lung in the region of each hilum with bilateral pneumothorax above and at the periphery. The shadow on the right is somewhat larger than that on the left, and is less well defined at its base, where there still remains a small portion of expanded lung. Motion of the diaphragm on both sides shows definite restriction, this being more marked on the left.

The gastrointestinal examination resulted in the diagnosis of gastric neoplasm, following the visualization of a prepyloric filling defect, opposite the palpable tumor previously mentioned.

Course in hospital: The patient was admitted to the hospital January 8, 1925, with pulse of 84, respirations 32, and temperature 97.8°. Because of his gastric disturbance, with a combination of clinical and physical symptoms suggesting neoplasm, an X-ray examination of his gastrointestinal tract was done on January 12, 1925, a report of which, together with one of the routine fluoroscopic chest examination, is included under laboratory tests.

He ran a fairly even course in the hospital until January 19th, when he quite suddenly developed a vague sense of discomfort, followed shortly afterward by increasing cyanosis and considerable dyspnoea and orthopnoea. He had hitherto been remarkably free from these symptoms. From this point his condition became continuously worse until on January 21st, with pulse of 124, respirations 44 and temperature 100.2°, it was obvious he was in imminent danger. At this time the cyanosis and the intense respiratory distress, with all the accessory muscles of respiration in use, presented a most distressing appearance.

He died shortly after midnight on January 22nd.

Permission for an autopsy was refused.

SUMMARY

This patient, with a bilateral pneumothorax discovered accidentally during a routine examination of the gastrointestinal tract, constitutes the ninth case of spontaneous bilateral pneumothorax in the literature. The etiological factor is not clear, as the entire clinical picture was obscured by the concomitant presence of carcinoma of the stomach.

We do not feel, however, that the latter disease had any bearing on the pulmonary condition. On the other hand, an autopsy having been refused, it cannot be said with finality that the condition was not brought on by pulmonary tuberculosis, especially since the expanded lung fields had not been examined, although from the law of probability this seems to be the most likely etiological factor. Without an autopsy, it is likewise hazardous to speculate upon the possible rôle played by the dusty atmosphere in which this man worked.

BIBLIOGRAPHY

MEYER, A.: *N. Y. Med. J.*, 1917, cv, 1238.
MARSHAK, M. I.: *J. A. M. A.*, 1918, lxx, 1518.
GRAY, E. A.: *Illinois M. J.*, 1919, xxxv, 252.
BOURNE, G.: *Brit. M. J.*, 1921, i, 526.
HAWES, J. B.: *Boston M. & S. J.*, 1922, clxxxvi, 528.
EBERTS, E. M.: *Arch. Surg.*, 1923, vi, 168.
EMERSON AND BEELER: *Am. J. Roentgenol.*, Feb. 2, 1923.
GRASHEY: *Fortschr. a. d. Geb. d. Roentgenstrahl., Kongressheft*, 1924, xxxii, pp. 40–41.

THE INJECTION OF THE SUPERIOR LARYNGEAL NERVE WITH ALCOHOL FOR THE RELIEF OF PAIN IN LARYNGEAL TUBERCULOSIS[1]

GEORGE I. SWETLOW

The question of laryngeal pain in pulmonary tuberculosis has been of such great concern that it has always enlisted the interest, aid and sympathy of especially those who come constantly in direct contact with this symptom. And although in most cases the amelioration of the distress does not mean eradication of the cause, any degree of relief that is obtained is nevertheless gratefully appreciated.

Of special importance is the pain seen in tuberculosis of the larynx. Not only is the pain so severe as to preclude the rest which is vital to a tuberculosis patient, but it also prohibits the ingestion of food because of the intense agony incurred on swallowing. Any procedure therefore that offers relief is gladly accepted by the patient.

It is interesting to note, according to the reports of various observers, that nearly one-third of all cases of pulmonary tuberculosis have shown, on necropsy, specific laryngeal lesions. Schwatt (1) who has collected 270 complete autopsies from the Montefiore Hospital, found 119 cases, or 44 per cent, with laryngeal tuberculosis. Nearly 65 per cent of laryngeal tuberculosis cases, according to Fishberg (2), complain of pain varying in severity.

The pain that is complained of has many attributes. The distress may or may not be associated with swallowing. The patients often describe it as dull or burning in character. Most often the pain is sharp and knife-like, and localized to the middle of the throat or in one or both sides of the neck. At times it radiates from the side of the neck to the homolateral ear. It is usually increased by the swallowing of foods, especially hot and spicy ones. In some cases only a little cool water or milk can be ingested. Coughing in most of the patients greatly aggravates the symptom. In some, even the swallowing of their saliva produces unbearable distress.

[1] From the Tuberculosis Service of the Montefiore Hospital for Chronic Diseases, New York City, Dr. M. Fishberg, Chief of Service.

In the search for relief many methods, varying from mild applications to radical surgery, have been tried with more or less success. I will briefly summarize them, with an attempt to convey the general consensus of opinion concerning the efficacy of each.

1. Laryngeal insufflations: Orthoform or anesthesin powder, 3 to 5 grains, is sprayed upon the ulcerations. It is applied one hour before meals and may give some temporary relief.

2. Inhalation of chloretone vapor: Chloretone crystals are heated and the vapor is inhaled or sucked into the larynx. This at times gives relief for as long as three hours in ulcerative cases.

3. Morphine: About $\frac{1}{4}$ grain is mixed well with 2 to 3 grains of starch powder and applied to the ulcerated area. Some relief is obtained. This is used only in terminal cases.

4. Cocaine: A five per cent aqueous solution is applied in late cases.

5. Submucous injections: A 2 per cent solution of novocain is injected into the swollen and infiltrated tissues. This is done once or twice daily. The relief obtained is only temporary.

6. Operative procedures:

a. Galvano-cautery and curettage: In Fishberg's experience (3) these methods have ended disastrously in all his cases.

b. Amputation of epiglottis: About 27 per cent of laryngeal tuberculosis cases show the epiglottis to be involved. Lockard (4), reporting 25 cases, claimed brilliant results. However, the mortality reported by Boldt (5) is too high to warrant such a procedure. Out of 55 cases, the latter lost 3 from postoperative hemorrhage, 2 from pneumonia and 2 from hemoptysis. The average period of relief was about one year. One case in the Montefiore Hospital was relieved for six months. Sokolowski of Warsaw is absolutely against this procedure because of poor results. In 10 cases Jorgen Moeller of Copenhagen had no results justifying so dangerous a method.

c. A third operative procedure is to cut down on the superior laryngeal nerve and either sever or crush it. Steinmann (6) reported 10 cases in which he resected the internal laryngeal branch with good results. Teller (7) in 12 cases obtained relief for a long time, only to have the pain reappear.

In discussing the operative results, Fishberg (8) emphatically states that he is absolutely opposed to any mechanical interference with the larynx. He feels that the duration of life is greatly shortened by such manipulations.

In 1908 Hoffmann suggested injecting the superior laryngeal nerve with alcohol for the relief of these patients. He reported several cases with good results. His method was taken up by other workers and the reports as to success are conflicting. Lukens has reported 19 cases in

which the shortest period of relief was three days and the longest period forty-one days after one injection. I wish to present 13 cases, with the idea of encouraging the further use of this procedure, as there has been a tendency to consider the method unsuccessful. I shall describe the technique used and attempt to point out the cause for obtaining indifferent results.

A clear comprehension of the anatomical course of the superior laryngeal nerve and the structures supplied by it is absolutely essential. The nerve arises from the lower ganglion of the vagus, running downward and inward beneath both of the carotid arteries toward the superior horn of the thyroid cartilage. About one centimetre in front of and a little below the cornu of the hyoid bone, the nerve divides into an internal and external branch. The internal branch enters the larynx by piercing the thyrohyoid membrane. It supplies the mucous membrane of the base of the tongue, epiglottis and the larynx. The external laryngeal nerve distributes its terminal filaments to the subglottic portion of the mucous membrane of the larynx and ventricle. It also gives off a few filaments to the pharyngeal plexus which supplies the constrictors of the pharynx. The ganglion of the trunk from which the superior laryngeal nerve arises is in anatomic connection with the upper ganglion of the vagus which gives off an auricular branch. After leaving the upper ganglion, which is situated in the jugular foramen, the auricular nerve courses along the inner surface of the facial canal and terminates between the mastoid process and the external auditory meatus. The nerve supplies the skin of the posterior part of the auricle and the posterior inferior portion of the external auditory canal.

The technique for injecting the superior laryngeal nerve has been described by several writers. The patient should be placed in the dorsal recumbent position with a rolled sheet placed beneath his shoulders. This throws the head back, and thus moderately stretches the soft structures of the anterior aspect of the neck. The great cornu on the side to be injected is made more prominent by pressing with the left forefinger the great horn on the opposite side. A needle, no. 2 (5 cm.), unattached to the syringe, is inserted about 2 cm. in front of the large horn of the hyoid and 1 cm. below. The needle is directed backward and slightly upward toward the great horn. The point of the needle must not be permitted to go laterally beyond a line joining the large cornu of the hyoid to that of the thyroid. Beyond this line the large blood-vessels of the neck are found. The point

is pushed in for about 1.5 cm. to lodge itself between the thyrohyoid muscle and the thyrohyoid membrane. We can be certain that the nerve has been struck only when the patient has a sharp pain in the ear on the side of the introduction. He indicates this not by talking but by raising his hand. It is indeed rare that the nerve is directly pinioned. This occurred only twice in my series. After reaching the desired spot the point is gently used as a probe to comb the entire thyrohyoid area. Only after the nerve is struck is the syringe attached to the needle and the solution injected. The patient is cautioned not to swallow while the injection is in progress. The solution is made up of 80 per cent alcohol (warm) to which cocaine hydrochloride, to make up 1 per cent of the total quantity, has been added. The cocaine is used with the idea of ameliorating the immediate local pain incident to the inflammatory action of the alcohol.

Before deciding to use the alcoholic form of treatment, a careful choice of the cases must be made. Those that have difficulty in swallowing not attributable to pain are to be excluded. An injection into the superior laryngeal nerve is under such circumstances unjustified anatomically and physiologically. The difficulty in swallowing under such conditions may be traced directly back to the pharyngeal muscles which are supplied through the pharyngeal plexus. The plexus is formed predominantly by the vagus and glossopharyngeal nerves. One should investigate these nerves and elicit the cause for this motor deficiency. The cases that indicate the injection of the superior laryngeal nerve are (1) those which have spontaneous pain without swallowing and (2) those thich have pain because of the ingestion of food. It is only by this analysis that the proper cases can be treated and the proper inferences drawn.

After deciding upon the case, the side to be injected must be chosen. The patient is the greatest aid. The injection is done on the side which is subjectively most painful. Often the more affected side is found by careful palpation. Greater sensitivity and tenderness are additional indications.

One of the most frequent reasons given for the failure to strike the nerve is that the nerve is anomalously placed. Neither Gray's nor Piersol's *Anatomy* notes this as a fact. Nevertheless, the explanation is conceivable. From the cases to be reported I wish to offer some of the more probable explanations. Only rarely is the nerve struck directly as the needle is introduced. To inject the solution without defi-

nitely entering the nerve is probably one of the chief reasons for failure. The thyrohyoid space is fairly large, so that the 2 cc. of the injected alcohol spreads along the fascial plane, and little, if any, is absorbed by the nerve. The thyrohyoid area must be probed lightly and patiently until the nerve is encountered. Little pain is felt once the point of the needle has passed through the skin. The syringe must not be attached to the needle before the nerve is found. It is clumsy and heavy and, as a result, excludes fine probing. The possibility of pharyngeal tuberculosis as a cause of pain is to be remembered. This condition may also explain some of the failures. Injections into the superior laryngeal nerve under such circumstances would not alleviate the pain.

In the following series of cases

1. Thirteen patients with advanced pulmonary tuberculosis and specific laryngeal tuberculosis were treated.

2. In one case there was complete failure to strike the nerve after several attempts were made.

3. Four cases that had agonizing pain and could swallow practically no food of any kind because of pain were completely relieved. One case has been free from all discomfort for twenty-two days. The pain has recurred, and after another injection the patient is again comfortable. Another has been absolutely free from all discomfort for forty-five days to date. Still another has been almost completely relieved for thirty-five days to date.

4. Another group of cases, though relieved of pain, have difficulty in "pushing the food down," as they put it. Various degrees of this difficulty are complained of. In fact, all of the cases treated had this motor disturbance which disappeared in a day or two. These few subjects still complain of this symptom. The apparent cause for this can be explained anatomically. The superior laryngeal nerve through its external branch contributes to the formation of the pharyngeal plexus, the latter of which supplies the constrictors of the pharynx. There were four such cases in this group. The degree of complaints has varied in intensity in the different patients. One has been relieved for forty-six days to date, another thirty-two days to date, another twenty days to date, and still another thirty-seven days to date. This last case is an exceedingly severe one. In fact, he has had his epiglottis surgically removed in another hospital because of the intense pain.

5. The third group also comprises four cases. The histories will more accurately describe their course.

6. Immediate effects after the injections are

a. Twelve cases complained of increased local pains lasting at most two days.

b. Nearly all cases complained of immediate motor difficulty in swallowing. Most of them cleared up in a few days.

c. In only two cases was there a slight rise in temperature the next day.

Case Reports

Case 1: S. E. Female. Age 22. For three months previous to the injection of her right superior laryngeal nerve, the patient complained of a constant soreness in the right side of her throat. This soreness was greatly aggravated by swallowing. Gradually the pain became more severe, sharp and knife-like. During the entire three months the patient had had great difficulty in swallowing fluids, solids or spicy foods, and had found it impossible to swallow warm foods. Consequently she ate very little. She had a far-advanced fibrocaseous pulmonary tuberculosis. The laryngeal examination showed the epiglottis to be greatly injected, oedematous and ulcerated on its free border. Both vocal cords were oedematous, the right more so than the left. Following the injection of alcohol her pain, both spontaneous and that that had been induced by food, completely disappeared. She could eat all foods, including spicy and those that are warm. This relief lasted for twenty-two days, but the pain reappeared. The nerve was reinjected and she is again comfortable, swallowing liquids and solids.

Case 2: S. G. Male. Age 30. For four months previous to the injection of the right superior laryngeal nerve, the patient complained of severe pain on the right side of the neck, which increased on swallowing. He could not swallow hot foods. Daily at 5 P.M. he would experience a severe, sharp pain in the throat that was agonizing in character. Physical examination showed a far-advanced pulmonary tuberculosis. The laryngeal examination disclosed that the epiglottis was swollen and ulcerated, that the right arytenoid was swollen and had a large mass present, that both vocal cords had small ulcerations, and that the ventricular bands were infiltrated. The right superior laryngeal nerve was injected with alcohol with the following results: All subjective pain has disappeared. The patient can swallow all kinds of food, including spicy hot food, with no pain at all. The agonizing pain that he experienced at 5:00 P.M. daily has disappeared. As a result of his being able to eat he feels stronger. It is now forty-five days that he is relieved of all discomfort.

Case 3: E. L. Male. Age 55. For two months before the injection of his right superior laryngeal nerve, the patient complained of severe, sticking pain in the right side of his throat, which was increased on swallowing solids, liquids, and spicy or hot foods. This pain prevented his eating. Physical examination showed a far-advanced chronic pulmonary tuberculosis. On laryngeal examination the right arytenoid appeared oedematous and the left one markedly infiltrated. Both vocal cords were infiltrated. Since the injection of the right superior laryngeal nerve the patient can swallow all types of food without pain. It is now forty-one days since he has been completely comfortable.

Case 4: D. N. Male. Age 35. For four months previous to the injection the patient had complained bitterly of pain in the right side of the throat, which was sharp, sticking and burning in character; that it shot through his right ear; that it gradually increased in intensity with practically no remissions; and that he was unable to swallow any form of food because of this pain, and he grew very weak as a result. Physical examination showed far-advanced pulmonary tuberculosis. On laryngeal examination the left arytenoid and left false vocal cord were found to be infiltrated. The right arytenoid was ulcerated and the intraärytenoid ligament had a coxcomb tuberculoma. After the injection all pain disappeared. The patient is able to eat all forms of food with only an occasional twinge. The pain in his right ear has completely vanished. Hot foods that he previously could not possibly swallow, now give him no pain. It is thirty-four days since he has been relieved of all discomfort.

Case 5: M. K. Male. Age 32. For eight months before the injection of the left superior laryngeal nerve the patient had had pain, at times more and at other times less, although some was always present. The pain was sticking, and was especially severe on the right side. It was so severe that he was unable to swallow any form of food. Physical examination showed far-advanced pulmonary tuberculosis. On laryngeal examination the epiglottis was found to be both infiltrated and ulcerated. Both arytenoids were infiltrated. A general infiltration of the entire larynx was present. Since the injection all pain in the throat has disappeared. The patient, however, complains of difficulty in "pushing the food down." He is able to swallow fluids, eggs, meats, tea and cereals. Coughing does not induce pain as it did before the treatment. It is now forty-six days that he has been comfortable and able to swallow.

Case 6: S. S. Male. Age 35. For eight weeks before the injection of the left superior laryngeal nerve the patient had a sharp and sticking pain in the throat with or without the act of swallowing. Swallowing any food or liquid greatly aggravated the pain, and the patient was entirely unable to swallow hot or warm fluids. Physical examination showed far advanced pulmonary tuberculosis. Laryngeal examination revealed an epiglottis that was markedly infiltrated and ulcerated, a right arytenoid that was swollen, and a right vocal cord that was immobile. Following the injection of the left superior laryngeal nerve all pain in the throat disappeared. For the first time in two months the patient was able to swallow fruits, cakes and hot fluids. He complained, however, of some difficulty in "pushing the food down." For thirty-two days he was greatly relieved. He then became weaker and died of tuberculosis.

Case 7: H. G. Male. Age 42. This patient had been complaining of severe pain and painful dysphagia for a year. The pain had been so severe that his epiglottis was surgically removed. He was relieved for a few months after this operation only to have the pain reappear with greater intensity. For seven weeks preceding his injection the pain had become so severe that he ingested practically no food. The pain was sticking, and so sharp that he could swallow only a little cool milk. Following the injection of the right superior laryngeal nerve, all pain in his throat disappeared, and pain that had been so agonizing on coughing vanished. For the first time in months he was able to swallow spicy soups and bread. He complained at first of motor difficulty in "pushing the food down," but this became much less troublesome. He had a total of twenty days of complete relief before he died of general pulmonary tuberculosis.

Case 8: J. G. Male. Age 67. For six weeks preceding the injection, the patient complained of a persistent, sharp, sticking pain in the left side of his throat, which radiated to the left ear. This pain was markedly aggravated by coughing, but was not appreciably increased on swallowing. Physical examination showed chronic pulmonary tuberculosis, far advanced. The laryngeal examination revealed that the entire larynx was congested and swollen and that the left vocal cord was infiltrated and immovable. Following the injection all pain completely disappeared, and coughing produced pain no longer. For thirty days he has been free from pain.

Case 9: S. W. Female. Age 23. For ten days before injection, the patient had complained of severe, sharp, sticking pain on the right side of the throat, which was increased by swallowing. The swallowing of solids and liquids was very difficult. Physical examination showed far-advanced pulmonary tuberculosis. The laryngeal examination revealed that the vocal cords were ulcerated and infiltrated, and that there were also ulcerations and infiltrations of the anterior surface of the larynx. Following the injection of the right superior laryngeal nerve, the pain disappeared, and the patient found herself able to ingest solids and liquids, and spicy as well as hot foods. She, however, complained of a "lump" on the dorsum of her throat. It is now forty-three days that she has been completely relieved.

Case 10: S. A. Male. Age 48. For ten months before the injection the patient com-

plained that he had severe, sticking pain on the right side of his neck and that the swallowing of solids, liquids and warm foods greatly increased it. Physical examination showed far-advanced pulmonary tuberculosis. On laryngeal examination there was found a marked infiltration of all the laryngeal structures. The epiglottis was apparently uninvolved. Following the injection practically all pain disappeared. The patient was able to swallow nearly all forms of food. Occasionally he has a slight, sharp twinge on swallowing. It is now forty-one days that he has been relieved.

Case 11: H. I. Male. Age 50. For two months before the injection the patient complained of a sharp, sticking pain on the left side of the throat, which pain was greatly increased on swallowing. He was unable to swallow solids, liquids, or hot and spicy foods. The pain was especially severe on coughing. Physical examination showed far-advanced pulmonary tuberculosis. Laryngeal examination revealed an epiglottis that was ulcerated and infiltrated, and two vocal cords that were greatly thickened. After the injection of the superior laryngeal nerve, the pain disappeared completely and the patient was able to swallow all foods. In fact he was able to drink coffee for the first time in eight weeks. It is now a total of twenty days that he has been relieved of all pain.

Case 12: D. C. Male. Age 66. For six weeks before the injection the patient complained that he had sharp, sticking pains on the left side of his throat, and that the pain was so intensified by deglutition that he could swallow no foods at all except small quantities of cool milk. Physical examination showed a very far-advanced pulmonary tuberculosis. The laryngeal examination disclosed the epiglottis to be both markedly infiltrated and ulcerated. Following the injection of his left superior laryngeal nerve, the pain disappeared and the patient was able to ingest cereals, bread and medication. He was comfortable and free from pain to the date of his death. He was relieved for a total of thirty days.

Note: The relief that is reported in the above cases is present to date.

SUMMARY

1. The injection of alcohol into the superior laryngeal nerve for the relief of pain is anatomically and physiologically a rational procedure.

2. It relieves pain for several weeks at least.

3. Difficulty in swallowing due to pain is often completely relieved.

4. Cases that have difficulty in swallowing, not due to laryngeal pain, are to be rejected for this treatment. One cannot expect results in this class of patients.

5. The procedure is free from danger and is practically painless.

6. The injection can be repeated if necessary.

7. This method has a definite and important rôle in the treatment of pain in tuberculosis of the larynx.

REFERENCES

(1) SCHWATT: Personal communication.
(2) FISHBERG: Personal communication.
(3) FISHBERG: Pulmonary tuberculosis, 3d Ed., p. 864.
(4) LOCKARD: Ann. Otol., Rhinol. & Laryngol., December, 1909.
(5) BOLDT: Dissert., Rostock, 1906.
(6) STEINMANN: Verhandl. d. 15. Kongr. Russischer Chirurgen, St. Petersberg, 1922, ix.
(7) TELLER: Ztschr. f. Tuberk., Bd. 36, H. 1, S. 35–36, 1922, xxxvi, 35.
(8) FISHBERG: Personal communication.

ARTIFICIAL PNEUMOTHORAX

A PLEA FOR ITS WIDER APPLICATION IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1,2]

I. D. BRONFIN, E. NELSON AND J. ZARIT

Collapse therapy is based on the broadest principle, now accepted as an axiom, that rest is of paramount importance in combating tuberculosis. It is no longer necessary to expatiate on the curative value of rest. The natural course of chronic phthisis, however, is characterized by long remissions, during which the patient is often enjoying such excellent health as to give him a sense of false security in the belief that an actual cure has been attained. That an actively progressive and ulcerative lesion can develop during such a period of remission without manifestations of strikingly acute symptoms, is a phenomenon with which every phthisiologist is familiar. It has been the experience of many to find an advanced bilateral and very extensive pulmonary involvement with a history of clinical symptoms of only three or four months' duration. The discrepancy between the history and the physical findings is usually explained on the assumption that the patient had been ill with tuberculosis for many years without being aware of its existence. This explanation is unquestionably plausible, but the problem as to what to do to prevent the outbreak of the disease remains unsolved. Rest, local or general, is at this advanced stage of little value or at best proves to be only palliative.

The Sanatorium of the Jewish Consumptives' Relief Society has treated in the last twenty years 4,926 patients. Of these, 61 per cent had had previous residences in institutions, and at the time of their discharge were considered quiescent and able to resume responsibilities of life. Over 90 per cent of the patients were classified upon admission as *far advanced*, and the mortality was 27.4 per cent among the men and 26.2 per cent among the women. These figures are appalling and bespeak a flaw in the modern sanatorium treatment of tuberculosis.

[1] From the Sanatorium of the Jewish Consumptives' Relief Society, Sanatorium, Colorado
[2] Read before the Denver Sanatorium Association, January 27, 1925.

General rest alone is manifestly not sufficient in a great many cases to cause an actual arrest of the disease. Quiescence in tuberculosis should therefore be looked upon as a smouldering ember which, under adverse conditions of life,—the usual plight of the average consumptive,—turns into an uncontrollable conflagration.

It has been stated that one of the reasons why pulmonary lesions fail to heal, or heal but slowly, is the constant distention of the lungs, rendering the coaptation of the diseased parts impossible. Recalling the additional familiar physiological fact that, as a result of the act of normal respiration, the lungs contract and expand about thirty thousand times a day, we begin to wonder how healing ever does take place.

It seems that surgeons were the first to appreciate the value of local rest to a diseased organ. The splint is one of the oldest mechanical appliances. The effect of motion or friction in disseminating infection from a suppurating focus had been observed by surgeons long before rest came to be recognized as a sovereign remedy in phthisis. It was probably for this reason that Murphy (1) became the staunchest advocate for administering pneumothorax in the early stages of the disease. He (2) emphasized his belief that the indications for pulmonary immobolization are the same as for the immobolization of surgical tuberculosis in any other portion of the body. His conclusion, based on a large clinical experience, was that the earlier the treatment is begun the better the prognosis.

With a better understanding of the pathology and evolution of pulmonary tuberculosis, it is all the more surprising to note the conservatism which many observers entertain with regard to the use of induced pneumothorax. Thus Boas (3), basing his opinion on experiences with advanced cases, believes that "the place which pneumothorax treatment should occupy in the treatment of tuberculosis is analogous to that which radium occupies in the treatment of cancer." Ringer (4), though somewhat more optimistic, sums up his attitude by saying that "the patient often gets a reprieve for a year or two, and that means a good deal." He, like the preceding author, had in his series chiefly advanced cases, and used collapse therapy as a palliative measure only. Nevertheless, 30 per cent of his 101 cases showed marked improvement and freedom from invalidism. Minor (5), though 12 of his 100 advanced cases were restored to working efficiency and 20 had their lives prolonged with comparative comfort from a few months to two years, criticized Murphy by saying that "if Murphy had been as good a

tuberculosis specialist as a surgeon, he would not have recommended its use in early cases." Minor evidently does not take into consideration the possibility that, if pneumothorax had been administered to his 44 unsuccessful cases during the earlier stages of the disease, he probably would not have found so many extensive pleural adhesions which, as he himself admits, were the chief causes for his failures.

Among the numerous American observers whose experience entitles them to definite views, we do not find any, with the exception of Murphy and Floyd (6), who advocate or approve collapse therapy for cases with unilateral pulmonary lesions in the absence of constitutional evidences of toxemia. The general trend of opinion seems to be that, as long as there are no active symptoms and the case is otherwise doing well, the pulmonary lesion *per se* requires observation only. Beggs (7), from an experience with 100 cases, thinks that earlier treatment by this method is very much to be desired, but hesitates to make such recommendation on account of the frequency of complications, chiefly pleural effusions; yet he is cognizant of the fact that pulmonary tuberculosis is not a "typical self-limited disease" and that the "trend of the advanced case is toward death with an ever-increasing rapidity." Barlow and Kramer (8), in discussing the indications for selective collapse, state that the method is suitable for those who are not beyond the hope of recovery but still are in need of additional assistance besides that afforded by the ordinary treatment. Whether one may infer from this that cases with stationary pulmonary lesions, even when ambulant and afebrile, should be subjected to this treatment is difficult to tell. Matson (9) and his associates, reporting on 423 cases with special reference to the behavior of the contralateral lung, state that all their patients received previous sanatorium treatment, many of them having been under observation for long periods of time, receiving all the benefits from climatic, dietetic and hygienic procedures. They considered this delay very unfortunate because, when they were "obliged to resort to an artificial pneumothorax," they were unable to introduce air on account of the presence of adhesions. From this statement one can see very clearly the dangers incident to delay in treatment. Although experience has taught some observers to institute collapse therapy much earlier than orthodoxy permits, it nevertheless seems that the afebrile and ambulant case, with no symptoms other than cough, expectoration and bacilli-laden sputum, is still permitted to shift for himself, or is allowed to "cure" in a sanatorium until discharged as "quiescent,"

only to return in due time with urgent indications for interference, and be informed that he cannot receive gas on account of adhesions.

This, in brief, is the present-day status of a remedy which is acknowledged by all to be superior to any other method thus far advanced in successfully coping with a disease, the tendency of which, if left alone, will in the majority of instances progress slowly or rapidly to a fatal termination.

If we accept as a correct procedure that a joint should be splinted as soon as a diagnosis of tuberculosis is established, there is no reason why measures similar in effect should not be instituted in a case of open pulmonary tuberculosis, even when limited to one or even part of one lobe. No one hesitates to recommend the removal of a tuberculous kidney even if only slightly diseased, because the dangers of spread of infection to the bladder and thence to the other kidney is clearly recognized, but unilateral pulmonary tuberculosis, though potentially if not actually as dangerous as the tuberculous kidney, is left alone, awaiting first the development of urgent symptoms.

This is not intended to minimize the value of general rest. We know that rest is capable of preventing a spread of disease and is a factor in promoting healing by fibrous tissue, but when we consider Bushnell's (10) rational explanation of the mechanical factor responsible for the spread of infection, a doubt arises as to whether we are justified in relying solely on general rest. The above author asserts that the diseased lung area, having lost some of its elasticity, expands less than the neighboring healthy portions, while at the same time it contains a greater amount of fluid from an increased circulation of lymph. It is thus possible that there is established a suction action of presumably contaminated lymph from the focus into the healthy lung tissue through the anastomosing lymph channels. This sounds plausible, and can readily account for the many cases which prove refractory to the most ideal sanatorium regimen, and progress from the incipient to the far-advanced stage of the disease. General rest alone evidently cannot stop the continuous expansion and contraction of the diseased lung, nor check the cough which taxes to further capacity the function of the diseased lung.

Illustrative of the views expressed above, the following cases are briefly cited:

F. C., case no. 4752, age 24, nurse, admitted January, 1921, with a history of insidious onset a year prior to admission with increasing weakness, cough, expectoration and positive

sputum. Physical examination disclosed a right upper-lobe infiltration. Clinical course was apparently favorable. After six months of observation, we considered her physically able to resume her work. She pursued her occupation under an ideal environment until April, 1924, when on account of low grade fever, frequent attacks of pleurisy, and increased cough and expectoration, she sought readmission. Physical examination at this time revealed a marked progression of the lesion, involving practically the entire right lung. Pneumothorax was attempted but no space was obtained on eight trials. As a further evidence of broken resistance, acute otitis media became manifest in October, and the purulent discharge showed tubercle bacilli almost in pure culture. What can now be offered to this patient? Even thoracoplasty is not indicated in view of the grave toxemia. The question naturally arises: Could this relapse have been prevented if pneumothorax had been instituted during her first residence?

J. L., case no. 4536, age 36, mechanic, admitted August 1, 1923, giving a history of insidious onset in 1921. Physical examination revealed infiltration of the left upper lobe and the right apex. During the first six months of residence, he had two febrile reactions up to 101°F. lasting two to three days. In the intervals, he seemed to enjoy a fair degree of health. Yet, realizing the danger, we advised pneumothorax; but the patient refused, as he was planning to return to France. A progression of the lesion steadily ensued with a corresponding increase in symptoms, pleuritic pains being a marked clinical feature. In November, 1924, several attempts were made to collapse the left lung, but no space was obtained. Whether pneumothorax could have been successfully administered when first offered is a question. The disadvantage of procrastination becomes apparent, however, in this case, as the repeated pleuritic attacks increased the tendency to failure because of the resulting adhesions.

T. C., case no. 4565, age 21, admitted September 14, 1923, with a history of sudden onset with hemoptysis three months prior to admission. Physical examination disclosed active involvement of the upper half of the left lung and an inactive apical fibrosis. During the first year of residence no constitutional symptoms were apparent. The patient was afebrile and gained in weight, although the frequently repeated physical and roentgen examinations failed to show any evidence of local improvement. Collapse therapy was therefore not seriously considered until July, 1924, when a brisk hemoptysis suddenly occurred. For the following three months the course was active and progressive. Pneumothorax was instituted in October, and although a complete collapse was obtained the clinical course was not appreciably affected, due to a reactivation of the contralateral lung.

The above report illustrates that hemoptysis, a grave possibility in every case of active pulmonary tuberculosis, is a potent factor in disseminating infection. From the testimony of the patient, as well as from physical signs, the bleeding came from the left side, and it is reasonable to suppose that, had collapse therapy been instituted earlier in the course, hemoptysis would not have occurred and a spread of the lesion could have been avoided.

S. K., case 4603, age 17, shipping clerk, admitted October 20, 1923, with a history of acute onset four months previous to admission. Physical examination showed an active right upper-lobe infiltration and an inactive left apical fibrosis. With the exception of two febrile reactions, each lasting two days, the patient has been pursuing an apparently favorable course, although the physical signs are unchanged and the sputum is still positive. A

so called economic recovery can be considered as already accomplished in this case, but the question arises: Shall this young man be permitted to reënter active life, thereby subjecting himself to inevitable relapses or shall pneumothorax be instituted in order to prevent such a relapse?

Our apparently radical attitude is strengthened by a personal experience with 143 patients treated by induced pneumothorax during the past five years. These cases have been carefully classified with reference to the duration of the pulmonary disease prior to instituting pneumothorax and the influence of this time factor upon the type of collapse, the frequency of complicating effusions, the occurrence of accidental or spontaneous pneumothoraces, and the clinical end-results. Arbitrarily, we have divided our cases into two groups. Group 1 includes all those with a duration of pulmonary disease not exceeding three years, while group 2 refers to all those whose disease exceeded

TABLE 1

Type of pneumothorax with reference to duration of pulmonary disease in a study of 143 cases

	GROUP 1		GROUP 2				TOTAL
Duration of disease	1 year or less	1 to 3 years	3 to 5 years	5 to 7 years	7 to 9 years	Over 9 years	
Complete collapse	9	23	6	3	1	1	43
Incomplete collapse	4	18	11	10	0	1	44
Pocketed space	1	12	8	9	4	4	38
No space	1	10	4	1	1	1	18
Total cases	15	63	29	23	6	7	143

that period of time. Reference to table 1 will show that, out of a total of 78 cases of group 1, a complete collapse was obtained in 32, or 41 per cent; whereas, of the remaining 65 in group 2, a complete collapse was present in only 11, or about 17 per cent. Further, of the 43 complete collapses in our entire series, 32, or 74 per cent, were in group 1, leaving 11, or only 25 per cent, in group 2. This clearly demonstrates that the likelihood of obtaining a complete collapse is inversely proportional to the duration of the pulmonary disease. In other words, the earlier pneumothorax is instituted, the better the chance for a complete compression.

Experience has shown that some of the factors which tend to vitiate results in collapse therapy are the so called incomplete pneumothoraces, failing to favorably influence the cough or expectoration to any extent, on account of uncollapsed cavities and the pocketed pneumothoraces

which usually do not modify either the constitutional or the local symptoms. In the total group of 143 cases, 82, or 54 per cent, were of either of the two types referred to above. This virtually means that even under the most favorable circumstances, disregarding such almost inevitable complications as effusions or pulmonary perforation, reasonably good results in advanced cases cannot be expected in more than 50 per cent of the cases. Adding to this the 12 per cent in which no space could be obtained, on account of complete obliteration of the pleural space, a total of over 60 per cent of partial or complete failures is apparent, and is due chiefly to mechanical causes. It is realized that in a period of three years innumerable pathological changes in the visceral and parietal pleurae take place, all tending to hinder successful collapse

TABLE 2

*Leading complications with reference to duration of pulmonary disease**

	GROUP 1		GROUP 2				TOTAL
Duration of disease	1 year or less	1 to 3 years	3 to 5 years	5 to 7 years	7 to 9 years	Over 9 years	
Effusions	4 (26.6%)	21 (33.3%)	13 (44.7%)	15 (65.2%)	3 (50.0%)	5 (71.4%	61
Spontaneous pneumothorax	1 (6.7%)	2 (3.1%)	3 (10.3%)	1 (4.3%)	2 (33.3%)	1 (14.3%)	10
Total complications	5	23	16	16	5	6	71
Total cases	15	63	29	23	6	7	143

* Percentage refers to total number of cases at bottom of each column.

treatment; yet, cold facts of statistics show that results were better during that comparatively late period than during that beyond three years' duration. Seventy-eight, or 54 per cent, in our series had the pulmonary involvement for three years or less, and of these, 46, or 59 per cent, showed some impediment to complete collapse, while of the remaining 65 cases in group 2, 54, or 83 per cent, revealed an inability to obtain complete compression. These figures clearly show that the longer the duration of the pulmonary lesion the greater the tendency to failures from purely mechanical causes.

Effusion constitutes another bugbear to the pneumothorax operator and a serious menace to the patient. Table 2 shows that approximately 42 per cent developed an effusion at some time during the course

of treatment, 25, or 41 per cent, being in group 1 and 36, or 49 per cent, in group 2. Compared to the number of cases in each group, the percentage is even more striking. Of the 78 cases in group 1, the 25 effusions bring the proportion to 37 per cent, while of the 65 in group 2 the 36 bring the proportion to 56 per cent. It thus appears that the frequency of effusions also bears a definite relation to the duration of the disease.

The incidence of spontaneous or accidental pneumothorax in the course of induced pneumothoraces has been discussed by one of us (11) in another publication. It is but necessary to reiterate that its frequency is greater than is generally supposed, and a great many of the effusions, particularly those of the purulent type, can be traced to this

TABLE 3

Clinical results with reference to duration of disease

	GROUP 1		GROUP 2				TOTAL
Duration of disease	1 year or less	1 to 3 years	3 to 5 years	5 to 7 years	7 to 9 years	Over 9 years	
Slight improvement	3	3	2	1	1	1	11
Marked improvement	2	12	6	5	2	0	27
Economic recovery	5	22	5	2	0	0	34
No modification of course	3	11	7	7	3	4	35
Progression	1	7	3	2	0	1	14
Death	1	8	6	6	0	1	22
Total	15	63	29	23	6	7	143

source. In our series this accident occurred ten times, 3 or 30 per cent being in group 1 and 7 or 70 per cent in group 2.

In analyzing clinical results, an arbitrary terminology has been resorted to, based upon both symptoms and physical signs. Patients whose fever became lower and cough and expectoration somewhat lessened are said to have slightly improved. Those whose temperature began to range within normal variations, associated with a considerable amelioration of the other symptoms, are termed markedly improved. Those who acquired a working ability of from four to six hours a day are termed recoveries. Progression has reference to aggravation of symptoms with increased involvement of the contralateral lung. Table 3 shows that economic recoveries occurred in 34, or 23 per cent. Of these, 27, or 79 per cent, were in group 1, and 7, or 2 per cent, in group 2. Marked improvement was noted in 27, or 19 per cent. Of these,

14, or 52 per cent, were in group 1 and 13, or 48 per cent, in group 2. In 11, or 8 per cent, there was only slight improvement, with 6, or 55 per cent, in group 1 and 5, or 45 per cent, in group 2. Thirty-five, or 25 per cent, failed to improve. Of these, 14, or 40 per cent, occurred in group 1 and 21, or 60 per cent, in group 2. Among progressive cases, 8, or 57 per cent, were noted in group 1 and 6, or 43 per cent, in group 2. Considering that in group 2 there were only 65 cases as compared to 78 in group 1, it is apparent that the percentage of progressive cases in group 2 was higher than appears from a superficial survey of these figures. Of a total of 22 deaths, 9, or 40 per cent, were in group 1 as compared to 13, or 60 per cent, in group 2. There can, therefore, be little doubt that clinical results, either amelioration of symptoms or actual economic recovery, depend largely upon the total duration of the pulmonary disease. The death rate, too, shows a relationship, the mortality being greater among those who had a longer period of illness prior to interference.

COMMENT

The literature on collapse therapy is now fairly replete, disclosing a few encouraging and many discouraging results. Boas (3) was unquestionably correct in his observations when, in summing up his results, he stated that a positive cure is rarely accomplished in advanced cases. A good collapse results in marked improvement for a time, and then some complication supervenes which causes the disease to resume its former course. While a reprieve for a year or two is perhaps all that can be expected from pneumothorax in such cases, is it not timely to consider pneumothorax as a curative instead of a palliative measure? "To achieve a sure success," Riviere (12) states, "the operation must be performed at the suitable moment," but the appropriate time has never been definitely pointed out. It is generally conceded that practically all advanced cases have passed through a stage when pneumothorax could have been successfully employed. Matson (13), basing his conclusions upon experiences derived from a study of 600 cases, concedes that the present-day practice of pneumothorax gives immediate results as regards mechanics in only two out of every five cases. Considering that end-results in the partially compressed cases were satisfactory in only 15 per cent of his series, and that in one-fifth of the total number he was unable to obtain a pleural space, it is obvious that the present status of collapse therapy is far from satisfactory.

Against the wider administration of pneumothorax in early cases many objections can be advanced, and the many complications already enumerated emphasized. The frequent occurrence of effusions, the occasional spontaneous pneumothorax, and the rare but most exasperating accidents of pleural shock and air embolism are ever cited as obvious dangers, yet, on careful analysis, each of these is shown to have a lesser incidence among early cases. The etiological factors responsible for accidental pneumothoraces, such as the rupture of a cavity or small tubercle overlying the visceral pleura or an emphysematous bleb in the same location, are less likely to exist in a case that has more or less of a circumscribed early lesion than in a widespread disease. Pleural shock and air embolism have been encountered in our series in a few cases, and all these occurred in those in which a satisfactory free space did not exist. The danger of contralateral lung involvement is lessened in early cases even when a recognizable lesion is present on the opposite side, because according to Graham (14) and Stivelman (15) the pleural cavity, from the pressure standpoint, has to be considered as one in subjects with a labile or flexible mediastinum. The somewhat increased intrapleural pressure, when not too great, has been shown to exercise a beneficial effect upon the contralateral lung.

A review of the histories of many advanced cases of pulmonary tuberculosis, which arrive annually in ever-increasing numbers from various Eastern cities to our sanatorium as a last haven of refuge, shows beyond doubt that the majority considered themselves recovered and fit to resume their former position in life at the time of leaving their previous sanatorium. There is no doubt that each of these sanatorium graduates received a thorough education in hygiene and sanitation and instructions how to live after entering into active life. Cognizance must, however, be taken of the numerous and almost insurmountable economic difficulties which these patients have to face. Assuming that they escape the dangers of relapse during the critical readjustment period, they know of no measures of how to prevent a gradual and unrecognized resumption of the morbid processes. Indeed, the disease may reassert itself insidiously, and, when active symptoms become manifest to the extent of producing complete physical disability, it is frequently too late to afford these sufferers other than temporary relief.

The case of the advanced consumptive, frequently presenting many extrapulmonary tuberculous complications, suggesting a widespread disseminating tuberculous disease, is an ugly reality which we are

compelled to face. The Eastern institutions treat these cases with apparent success, and we treat the same cases five or ten years later. It is for this reason that we make a plea for early pulmonary immobilization. We feel that every case, not excluding the one pronounced *quiescent* or *arrested* on discharge, should be warned of the dangers of relapse. Each patient should be told in unequivocal language that he harbors within his body a disease not unlike a malignant growth. He should be urged that frequent and oft-repeated examinations are the only methods whereby to ascertain the presence or absence of a recrudescence of the disease. The physician should also make it clear that a reappearance of symptoms may necessitate more radical measures than a repetition of the "rest" cure. We feel that pneumothorax should be instituted in every suitable case which sustains more than one relapse during the first year of postsanatorium life. It is possible that by paying more attention to the so called stagnant cases while in the sanatorium, and by supervising medically every sanatorium graduate, so that effective steps may be taken at the proper time, the number of hopeless consumptives would become gradually diminished.

CONCLUSIONS

1. The weakest point in the present-day practice of artificial pneumothorax is its late application.

2. The longer the duration of the pulmonary lesion, the greater the tendency to unsatisfactory mechanical results.

3. The incidence of complications, such as effusions and pulmonary perforations, is greater in the advanced than in early cases.

4. The chances for economic or actual recovery are better when pneumothorax is instituted comparatively early in the disease.

REFERENCES

(1) MURPHY, J. B.: Surg. Clinics, December, 1913, vi.
(2) MURPHY, J. B., ET AL.: Interst. Med. J., 1914, xxi, 3.
(3) BOAS, E. P.: N. Y. Med. J., 1921, cxiii.
(4) RINGER, P. H.: Tr. Nat. Tuberc. Assn., 18th Ann. Meeting, 1922.
(5) MINOR, C. L.: Amer. Rev. Tuberc., 1917, i, 522.
(6) FLOYD, C.: Boston M. & S. J., 1918, clxxxviii, 2.
(7) BEGGS, W. N.: Amer. Rev. Tuberc., 1917, i, 509.
(8) BARLOW, N., AND KRAMER, D.: Trans. Nat. Tuberc. Assn., 18th Ann. Meeting, 1922
(9) MATSON, R. W., MATSON, R. C., AND BISAILLON, M.: Ibid., 1924, p. 160.
(10) BUSHNELL, G. E.: Amer. Rev. Tuberc., 1918, ii, 259.

(11) BRONFIN, I. D.: Ibid., June, 1924, ix, no. 4.
(12) RIVIERE, C.: Pneumothorax treatment of tuberculosis, Oxford Med. Press.
(13) MATSON, R. W., MATSON, R. C., AND BISAILLON, M.: J. Am. M. Assn., September, 1923, lxxxi.
(14) GRAHAM, E. V., AND BELL, R. D.: Am. J. Med. Sci., 1918, clvi, 839.
(15) STIVELMAN, B. P., HENNEL, H., AND GOLEMBE, H.: Trans. Nat. Tuberc. Assn., 18th Ann. Meeting, 1922, p. 161.

ARTIFICIAL PNEUMOTHORAX

RESULTS AT THE MUSKOKA INSTITUTIONS OF THE NATIONAL SANITARIUM ASSOCIATION[1]

WALTER B. KENDALL

The classification of the American National Tuberculosis Association is used throughout.

Percentages approximate to 0.5 per cent.

From 1914 to March, 1925, treatment has been attempted in 443 cases. Of these, 331 received complete, partial satisfactory, or unsatisfactory compressions, and in 112 the pleural space has proved to be totally inaccessible.

A complete compression has been considered as one in which the lung may show a few adhesions but the physical and roentgenographic evidence indicates a complete suspension of its function of aeration.

A partial satisfactory compression is one in which the greater part of the pulmonary parenchyma is thrown out of function, the influence being marked chiefly on the most badly diseased parts.

An unsatisfactory compression is one in which there appears to be no appreciable restraint on the function of aeration. In the *no compression* cases there has been either no evidence of gas in the pleural space or the pocketing has been small.

To demonstrate the value of pneumothorax treatment, the *no compression* cases are used as the most suitable controls. These patients are essentially the same type of cases as those in whom compression was obtained. With very few exceptions, all have been cases presenting definitely unfavorable or very guarded prognoses.

In table 2 the ultimate results in all cases of compression, complete, partial, satisfactory or unsatisfactory, are contrasted with those obtained by general treatment in *no compression* cases.

Summarized on the basis of improvement or nonimprovement, the contrast is increased, as shown in table 3.

This shows an increase on the side of favorable prognosis of 30 per cent in all cases able to receive any treatment at all.

[1] Gravenhurst, Ontario.

TABLE 1

		per cent			per cent
Treated	331	74.5	Complete compression	99	22.5
			Partial satisfactory	164	37.0
			Unsatisfactory	68	15.0
No compression	112		No compression	112	25.5
	443			443	

TABLE 2

	TREATED		NO COMPRESSION	
		per cent		per cent
Apparent cure	18	5.5	0	0.0
Arrest	37	11.0	3	2.5
Apparent arrest	34	10.5	1	1.0
Quiescent	34	10.5	10	9.0
Improved	70	21.0	18	16.0
Unimproved	20	6.0	24	21.5
Died	118	35.5	56	50.0
	331		112	

TABLE 3

	TREATED		NO COMPRESSION	
		per cent		per cent
Improved, etc.	193	58.5	32	28.5
Unimproved	138	41.5	80	71.5
	331		112	

TABLE 4

	THERAPEUTIC COMPRESSION		UNSATISFACTORY AND NO COMPRESSION	
		per cent		per cent
Apparent cure	18	7.0	0	0.0
Arrest	35	13.5	5	3.0
Apparent arrest	32	12.0	3	1.5
Quiescent	34	13.0	10	5.5
Improved	54	20.5	34	19.0
Unimproved	16	6.0	28	15.5
Died	74	28.0	100	55.5
	263		180	

But those listed as *Treated* include 68 patients whose compression was unsatisfactory; that is to say, their compressions were of such limited amount and the treatment of such short duration, that the mechanical effect of the therapy was a negligible factor in the progress of the case. (It will be shown later that the results in those cases are identical with the results in the *no compressions*.) To show the results of therapeutic amounts of compression in proper perspective, the cases of *unsatisfactory compression* are now listed with those of *no compression* (table 4), summarized on the basis of improvement or nonimprovement (table 5).

TABLE 5

	THERAPEUTIC COMPRESSION		UNSATISFACTORY AND NO COMPRESSION	
		per cent		*per cent*
Improved, etc.	173	66.0	52	29.0
Unimproved, etc.	90	34.0	128	71.0
	263		180	

TABLE 6

	COMPLETE		PARTIAL		UNSATISFACTORY		NO COMPRESSION	
		per cent		*per cent*		*per cent*		*per cent*
Apparent cure	10	10.0	8	5.0	0	0.0	0	0.0
Arrest	19	19.5	16	10.0	2	3.0	3	2.5
Apparent arrest	19	19.5	13	8.0	2	3.0	1	1.0
Quiescent	15	15.0	19	11.5	0	0.0	10	9.0
Improved	16	16.0	38	23.0	16	23.5	18	16.0
Unimproved	3	3.0	13	8.0	4	6.0	24	21.5
Died	17	17.0	57	34.5	44	64.5	56	50.0
	99		164		68		112	

This shows an increase on the side of favorable prognosis in those who were able to receive gas in therapeutic quantities of 37 per cent. In other words, without artificial pneumothorax an average case had 29 chances out of 100 of improvement. Upon receiving the treatment his chances increase to 66.

These figures are further modified in a way that it is impossible to show in statistics, by the fact that of those who received treatment and ultimately failed or died, 60, or 43.5 per cent, showed a temporary gain with abatement of symptoms and life evidently prolonged to a variable degree. Tables 4 and 5 show best the ultimate results of therapeutic compression.

The effect of the degree of compression obtained is shown in table 6.

Summarized on the basis of improvement or nonimprovement, the data appear as in table 7.

Table 7 shows almost identical results in the cases of *no compression* and the *unsatisfactory compression* cases, and shows, on the contrary, an increase on the side of favorable prognosis of over 50 per cent in cases of *complete compression* and of 30 per cent in cases of *partial satisfactory compression.*

TABLE 7

	COMPLETELY COMPRESSED		PARTIAL SATISFACTORY		UNSATISFACTORY		NO COMPRESSION	
		per cent		*per cent*		*per cent*		*per cent*
Improved, etc.	79	80.0	94	57.5	20	29.5	32	28.5
Unimproved, etc.	20	20.0	70	42.5	48	70.5	90	71.5

TABLE 8

	MINIMAL				MODERATELY ADVANCED				ADVANCED				ALL
	Treated		No compression		Treated		No compression		Treated		No compression		No compression
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	*per cent*
Apparent cure	1	5.0	0	0.0	8	4.0	0	0.0	9	7.0	0	0.0	0.0
Arrest	3	14.0	0	0.0	25	13.0	2	4.0	9	7.0	1	2.0	2.5
Apparent arrest	4	19.0	0	0.0	20	11.0	1	2.0	10	8.0	0	0.0	1.0
Quiescent	3	14.0	1	20.0	22	12.0	5	9.0	9	7.0	4	8.0	9.0
Improved	5	24.0	1	20.0	48	26.0	11	20.0	17	14.0	6	12.0	16.0
Unimproved	1	5.0	0	0.0	14	7.0	14	25.0	5	4.0	10	18.0	21.5
Died	4	19.0	3	60.0	51	27.0	22	40.0	63	53.0	31	60.0	50.0
	21		5		188		55		122		52		

Table 8 shows the influence of extent of disease on results. This is summarized in table 9. The treated cases include unsatisfactory compressions. A relatively small number of minimal cases have been attempted, 26 out of 443. Of the 21 whose pleural space has been accessible to some degree, 76 per cent have shown improvement contrasted with 40 per cent in the controls. Of the moderately advanced type, 66 per cent of the treated cases and 35 per cent of the controls have shown improvement. In the far-advanced type 43 per cent have shown im-

TABLE 9

Summarized on the basis of improvement or nonimprovement

	MINIMAL				MODERATELY ADVANCED				ADVANCED				ALL
	Treated		No compression		Treated		No compression		Treated		No compression		No compression
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	*per cent*
Improved, etc.	16	76.0	2	40.0	123	66.0	19	35.0	54	43.0	11	22.0	28.5
Unimproved	5	24.0	3	60.0	65	34.0	36	65.0	68	57.0	41	78 0	71.5

TABLE 10

Degree of toxic symptoms

	LIGHT				MODERATE				SEVERE				ALL
	Treated		No compression		Treated		No compression		Treated		No compression		No compression
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	*per cent*
Apparent cure	6	6.0	0	0.0	7	3.5	0	0.0	5	10.0	0	0.0	0.0
Arrest	14	16.0	1	4.0	20	10.5	2	2.5	3	6.0	0	0.0	2.5
Apparent arrest	13	14.0	0	0.0	19	10.0	1	1.5	2	4.0	0	0.0	1.0
Quiescent	10	10.5	2	8.0	22	12.0	7	10.0	2	4.0	1	6.0	9.0
Improved	19	20.0	8	32.0	42	22.5	9	13.0	9	18.0	1	6.0	16.0
Unimproved	6	6.0	5	20.0	11	5.0	16	23.0	3	6.0	3	17.5	21.5
Died	26	27.5	9	26.0	66	35.5	35	50.0	26	52.0	12	70.5	50.0
	94		25		187		70		50		17		

TABLE 11

	LIGHT				MODERATE				SEVERE				ALL
	Treated		No compression		Treated		No compression		Treated		No compression		No compression
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	*per cent*
Improved, etc.	62	66.5	11	44.0	110	58.5	19	27.0	21	42.0	2	12.0	28.5
Unimproved	32	33.5	14	56.0	77	41.5	51	73.0	29	58.0	15	88.0	71.5

provement, contrasted with 22 per cent of this class in the controls and 28 per cent of all *no compression* cases.

Table 10 shows the influence of the acuteness of disease, expressed in degree of toxic symptoms, on the results. This is summarized in table 11 and indicates, of course, that prognosis is better when the toxemia is light, showing 66.5 per cent improvement in this type, 58.5 per cent improvement in the moderately severe type and 42 per cent in the most severe types.

The improvement of 66.5 per cent in cases of light toxemia contrasts with 44.0 per cent improvement in this type of controls; 58.5 per cent

TABLE 12

	TREATED				NO COMPRESSION			
	Unilateral		Bilateral		Unilateral		Bilateral	
		per cent		*per cent*		*per cent*		*per cent*
Cured	8	5.0	10	6.0	0	0.0	0	0.0
Arrested	25	15.0	12	7.5	2	4.0	1	1.5
Apparent arrest	20	12.0	14	8.5	1	2.0	0	0.0
Quiescent	21	12.5	13	8.0	4	8.5	6	9.5
Improved	37	22.0	33	20.0	8	16.5	10	15.5
Unimproved	10	6.0	10	5.0	12	25.0	12	19.0
Died	47	27.5	71	44.0	21	44.0	35	54.5
	168		163		48		64	

TABLE 13

	TREATED				NO COMPRESSION			
	Unilateral		Bilateral		Unilateral		Bilateral	
		per cent		*per cent*		*per cent*		*per cent*
Improved, etc.	111	66.5	82	50.0	15	31.0	17	26.5
Unimproved	57	33.5	81	50.0	33	69.0	37	73.5

improvement in cases of moderate severity as against 27.0 per cent in this type of controls; and 42 per cent improvement in severe cases as against 12.0 per cent in this type of controls.

Fluid accumulated in 84 cases, or 28.8 per cent of those receiving treatment.

Purulent empyema developed in 6 cases, or 1.5 per cent of those treated.

Spontaneous collapse following refills occurred in 3 cases, or 0.75 per cent of those treated.

The 443 cases treated have received a total of 9602 fills.

The average duration of treatment in this summary of cases has been two years.

Cured	3 years 10 months
Arrested	2 years 10 months
Apparently arrested	3 years 3 months
Quiescent	2 years 1 month
Improved	1 year 4 months
Unimproved	9 months
Died	1 year 1 month

TABLE 14

	NATIONAL SANITARIUM		RIVIERE'S SUMMARY	
	All cases treated	Complete and partial satisfactory	American	European
	per cent	*per cent*	*per cent*	*per cent*
Arrested and cured	16.5	20.5	10.9	16.1
Quiescent (N. S. A. quiescent and apparently arrested)	21.0	25.0	10.8	9.8
Improved	21.0	20.5	29.2	31.2
Unimproved and died	41.5	34.0	49.1	42.9

TABLE 15

	NATIONAL SANITARIUM		RIVIERE'S SUMMARY	
	All cases treated	Complete and partial satisfactory	American	European
	per cent	*per cent*	*per cent*	*per cent*
Improved, etc	58.5	66.0	50.9	57.1
Unimproved and died	41.5	34.0	49.1	42.9

Of the 443 cases attempted, 216 were unilateral and 227 were bilateral, respectively 49.0 per cent and 51.0 per cent.

Summarized on the basis of improvement or nonimprovement; the results are as in table 13.

This shows improvement in the unilateral cases of almost 35.0 per cent more than in the same type of controls. The improvements in the bilateral cases exceed those in the bilateral controls by 23.0 per cent and even exceed the improvements in the unilateral controls by 19.0 per cent.

A comparison of our statistics with Riviere's summary of American

and European results more than confirms his statements of the value of artificial-pneumothorax therapy in selected types of cases (table 14).

When summarized on the basis of improvement or nonimprovement, results are as in table 15.

What makes our results appear superior in these tables is probably the fact that our figures are for one institution, where we have been able to keep, to a large extent, our patients under close observation and good regimen for extended periods; whereas Riviere's summary is the general average of many institutions, including several that, because of adverse economic influences and other reasons, are less fortunate in this respect.

A THORACIC SPLINT FOR PULMONARY TUBERCULOSIS[1]

W. C. HOWELL[2]

The accumulation of a considerable number of patients in our Sanatorium, in whom we could not induce artificial pneumothorax, or who would not accept it, caused me to undertake the task of finding a substitute that would accomplish something like the results obtained from pneumothorax.

The treatment of tuberculosis of any part of the body is based on the principle of rest or immobilization. Every definite advance in the treatment of tuberculosis for the last forty years has utilized the principle of functional rest, either directly or indirectly.

Tuberculous lesions of the lungs heal slowly because of the difficulty of resting the lungs. Motion of the lungs tends to prevent healing, while rapid breathing or laborious breathing causes the absorption of large quantities of toxins.

Krause has set forth clearly the importance of local as well as general rest in his book entitled *Rest and Other Things*. He says that of all the remedies proposed in the treatment of tuberculosis, only rest has stood the test of time.

Webb (1) says, "In breathing, a normal person opens and shuts the lungs nearly thirty thousand times a day. By rest we aim to make the breathing as shallow as possible, imitating almost that of hibernating." Any measure that limits the motion of the diseased lung is to be hailed with delight. It is quite apparent that surgeons are using the knife less in tuberculous lesions, and are resorting more to rest.

Artificial pneumothorax has been a great addition to our armamentarium, but there are certain cases on whom we cannot use this measure because of pleural adhesions. These cases are then subjects for postural rest, rib resection, or phrenic-nerve resection, or a combination of these measures. The outlook is not good for those who need pneumothorax badly and on whom it cannot be used. There are also patients who have an intense fear of pneumothorax and will not consent to its use.

[1] From Star Ranch-in-the-Pines Sanatorium.

[2] Colorado Springs, Colorado.

Many patients have rejected pneumothorax because of the dread of the use of needles, and would accept a less formidable looking procedure. There are again those patients who cannot get pneumothorax after returning home except at great inconvenience and large expense, and on these one hesitates to start pneumothorax until the way seems clear.

Postural rest is a very tiresome procedure when practised the greater part of the twenty-four hours, but if some other means of immobilization is used during the day the patient can easily sleep on the more affected side at night. Intensive postural rest causes painful callosities of the ear, soreness and pain about the shoulder, and a constant sense of restraint that, taken together, are not trifling matters.

Rib resection is an incomplete measure, even though it is an extensive operation, and unless some means of immobilizing the side be used for a year after the operation the results may be disappointing. Straps made of adhesive plaster and elastic bands have been used for this purpose, but they require frequent changing, do not exert uniform pressure, and irritate and denude the skin. Some people cannot tolerate them long.

Phrenic nerve resection is regarded by many as only an adjunct to more efficacious measures, and has not stood the test of time.

Jackets and casts, heretofore devised, are usually very uncomfortable, are more or less expensive, and restrict the movement of the better lung, the lung that should carry on the greater part of the work. When this is done, the more diseased lung is not getting the desired amount of rest or fixation. Most of these jackets bind the better side as much as the more diseased side, and the principle seems wrong. It is folly to strap up a patient in such a jacket and expect him to carry out the rest cure when he has to work for every breath he gets.

The molded splint to be described is being used with much satisfaction on the following types of cases:

1. Those who have had rib resection to produce collapse of the lung.
2. Those on whom artificial pneumothorax cannot be induced.
3. Those who fear the use of pneumothorax, or who cannot get it continued practicably after returning home.
4. Those who are not far enough advanced to warrant the use of pneumothorax, but do not respond quite satisfactorily to the rest cure.

CONSTRUCTION OF THE SPLINT

The thoracic splint is made by using ordinary plaster-of-Paris bandages to mold a splint to the front and back of the affected side. These splints are entirely separate, and should have a space of about two inches up and down the midaxillary line. The splints should extend from just below the clavicle in front and just above the spine of the scapula behind to below the ribs, front and back (fig. 1). Instead of separate splints being made, a jacket covering the whole side, almost to the median lines, front and back, can be made at once, and a two-inch strip removed from the axillary area to make the two separate splints. When the splints are about half-made, and just strong enough

FIG. 1.

FIG. 2.

FIG. 1. SHOWING THE LENGTH OF THE SPLINT AND THE THREE SPRINGS
FIG. 2. ONE OF THE SPRINGS CUT FROM A PHONOGRAPH SPRING

to stand a moderate amount of pressure, three springs are placed over the splints, and over these are placed more layers of the plaster-of-Paris bandages to incorporate the springs, cover the springs, and fix them. These springs can be cut from the innermost part of a discarded phonograph spring (fig. 2), and should be from ten to thirteen inches long, depending upon the size of the patient. Care should be taken not to make the appliance unnecessarily thick, and it should not reach quite to the sternum in front, nor the spines of the vertebrae behind. After drying an hour it can be removed, trimmed, and the edges smoothed.

INSTRUCTIONS TO PATIENTS

The patient should be instructed to wear the appliance for only a half-hour at a time, for several times the first day. Each day he can wear it for a longer and longer period until it is worn twelve or more hours a day. It can be easily worn while in bed, and the patient should always put it on before leaving the bed, in order to allow the fine filaments of scar tissue to be formed and to be not broken each day by rapid or laborious breathing.

We can understand this scar tissue better if we note what Lawrason Brown (2) says: "At first the scar tissue is very, very delicate, possibly

FIG. 3. LATERAL VIEW, TO SHOW THAT SPLINT IS SELF-SUPPORTING

no stronger than a spider web. A deep breath, a sudden exertion which causes us to shut our windpipe and contract our chest muscles, may readily stretch or break this scar tissue and so allow the germs to escape."

One of the benefits of this splint comes from the influence it has on the act of coughing. One coughs less while wearing the splint, and the coughing is not so forceful or injurious, if coughing be injurious. Sputum finds its way up without so much coughing in those patients who learn to suppress their coughs.

It seems thoroughly in keeping with long-established surgical princi-

ples, that the lung should heal more quickly if sputum were not retained but readily drained from the lung. Retention of pus and bacteria and their products has not been tolerated in other diseases and other parts of the body, and why should we encourage it in tuberculosis? This is just what we do when we employ postural rest. When one lies for many hours on the more affected side the sputum is retained for a greater part of the time, or a certain residuum is held back. When a patient wears this splint he can lie on the better side and drain the more diseased lung, and, at the same time, greatly limit the motion in the more diseased lung.

SUMMARY

The thoracic splint is a device that can be made at the bedside of the patient by the attending physician, and is made of inexpensive materials.

This splint can be made to fit the individual patient perfectly, and with as little or as much tension as desired.

It is suitable for a large number of tuberculous subjects, and can be used as an aid to other measures.

It can be worn in bed, and clings snugly to the patient when sitting up or walking around.

With but few exceptions (5 per cent of the cases) our patients keenly appreciate the splint, wear it from early morning until bedtime, and feel more comfortable with it on than off. After wearing the splint for several months, a decided restriction in the motion of the diseased side is noticed, even when the splint is removed.

REFERENCES

(1) WEBB, G. B.: Pulmonary tuberculosis, Fishberg, 1916, p. 471.
(2) BROWN, L.: Rules for recovery from tuberculosis, p. 26.

THE EFFECT OF THE ORAL ADMINISTRATION OF CALCIUM LACTATE AND THYROID EXTRACT ON THE CALCIUM CONTENT OF THE BLOOD SERUM IN PULMONARY TUBERCULOSIS[1, 2]

MAX M. TEPLITZ

Many experimenters have given calcium salts orally and noted the effect upon the calcium content of the blood. Few observations have been made along this line in pulmonary tuberculosis.

Muggia (1) was unable to raise the calcium content of the blood by giving inorganic calcium salts. Mason (2) found that 5 gm. of calcium chloride raised the plasma calcium 2.5 mgm. per 100 cc. in three hours, but he obtained no effect with calcium lactate. Denis and Minot (3) could not increase the blood calcium of normal persons given 6 gm. of calcium lactate per day from six to ten days. Howland and Marriott (4) found that feeding 0.5 to 1 gm. of calcium chloride to children with tetany, which is accompanied by a marked fall in the serum-calcium content, caused a rise in the blood calcium, sometimes to normal figures. Jacobowitz (5) could not increase the serum-calcium content in tetany by giving calcium salts, even when therapeutic results were excellent. Matz (6) increased the blood calcium in normal and tuberculous subjects by feeding either calcium lactate or calcium chloride. The latter salt caused a more marked rise. This author also observed that tuberculous patients receiving codliver oil and calcium lactate showed a greater and more sustained blood calcium increase than the same patients taking calcium lactate alone.

Maver and Wells (7) claim that

> it is to be expected that figures lower than normal can be raised to normal, but that higher figures are not to be obtained, since we have it well shown by the phenomenon of metastatic calcification that the normal blood calcium represents about the maximum amount that the blood can carry, since greater

[1] Read at the monthly meeting of the medical staffs of the Connecticut State Tuberculosis Sanatoria, February, 1925.

[2] From the Laboratory of the Hartford State Tuberculosis Sanatorium, Hartford, Connecticut.

amounts are promptly precipitated out in those tissues where the alkalinity is greatest or the CO_2 content the least.

Novak and Hollender (8) were able to increase the serum-calcium content of certain cases of hay fever and hyperesthetic rhinitis, which showed a low calcium figure, by giving orally calcium lactate together with thyroid extract. But they could not obtain these results by giving calcium lactate or thyroid extract alone. These authors conclude that "It is evident that the thyroid extract (or parathyroid) plays an important part in (1) stimulating the calcium metabolism of the body, or in (2) activating the artificial calcium in addition thereto and fixing it so that it is not immediately excreted."

The present investigation is similar to that of Novak and Hollender but is applied to pulmonary tuberculosis. Sixty patients were chosen, in all stages of the disease and of all degrees of activity. These cases were divided into six groups of 10 each.

Group 1 received calcium lactate, gr. v, and thyroid extract, gr. $\frac{1}{4}$, after meals for six weeks. Groups 2 and 6 were given calcium and thyroid for three months. Group 3 received calcium alone for three months. Group 4 was given thyroid alone for three months. Group 5, used as a control, was given a placebo tablet after meals.

Groups 1 to 5 comprised quiescent, mildly active, and moderately active cases. Group 6 consisted of markedly active and very toxic patients.

Serum-calcium determinations were made on each patient before treatment and three months later, at the end of treatment. In group 1 determinations were made after six weeks and after three months. At each period two determinations were completed on each patient at intervals varying from four days to two weeks. The average was taken as the correct reading. In group 6 only one determination was made at each period.

The method used in estimating the calcium content of the serum was that of Kramer and Tisdall (9), called *A Clinical Method for the Quantitative Determination of Calcium and Magnesium in Small Amounts of Serum or Plasma.* This was found to be a rapid and comparatively accurate method. Only 2 cc. of serum were required for each determination. In every instance the blood was withdrawn from the arm two and one-half to three and one-half hours after the midday meal. All the patients were on the same diet, the ordinary mixed house diet with an abundance of milk.

The findings are summarized as follows: The group average of those receiving calcium and thyroid for six weeks was 10.2 mgm. of calcium per

TABLE 1

Group 1. Calcium and thyroid discontinued after six weeks

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		BEFORE TAKING			AFTER 6 WEEKS' CALCIUM AND THYROID TREATMENT			AFTER 3 MONTHS. TREATMENT DISCONTINUED 6 WEEKS BEFORE		
		Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum		
		October 18	October 26	Average	December 3	December 10	Average	January 22	January 27	Average
C. B.	F. A. C.	9.4	10.1	9.7	9.5	9.5	9.5	9.3		9.3
A. T.	M. A. Q.	10.3	10.8	10.5	10.3	10.3	10.3	Left institution		
J. McB.	F. A. C.	10.1	9.2	9.6	9.8	9.9	9.8	9.6	9.2	9.4
G. H.	F. A. A.	10.0	9.8	9.9	9.9	10.2	10.0	9.9	10.0	9.9
C. W.	M. A. A.	11.3	10.2	10.7	10.3	9.9	10.1	9.6	10.6	10.1
F. M.	M. A. A.	9.6	10.3	9.9	10.4	9.5	9.9	10.1	10.4	10.3
C. H	M. A. B.	10.8	10.8	10.8	10.3	10.2	10.2	10.1	10.1	10.1
T. F.	M. A. Q.	10.8	10.0	10.4	9.7	10.0	9.9	10.1	9.8	10.0
Average				10.2			10.0			9.9

TABLE 2

Group 2. Calcium and thyroid for three months

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		BEFORE TAKING			AFTER THREE MONTHS		
		Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum		
		September 26	October 12	Average	January 20	January 24	Average
J. K.	F. A. A.	9.8	10.1	10.0	9.9	10.6	10.2
F. F.	F. A. Q.	10.7	10.0	10.3	10.8	10.4	10.6
V. G.	F. A. B.	9.9	10.2	10.0	9.6	9.8	9.7
J. R.	F. A. B.	10.6	9.8	10.2	10.2	9.8	10.0
J. S.	F. A. B.	10.2	9.5	9.8	9.8	10.5	10.1
A. G.	F. A. A.	10.6	10.2	10.4	11.2	10.8	11.0
R. M	M. A. Q.	10.0	10.7	10.3	10.8	10.5	10.6
M. O'C.	F. A. A.	9.9	10.0	9.9	10.8	9.8	10.3
J. B.	M. A. Q.	10.0	10.4	10.2	10.8	10.2	10.4
P. B.	M. A. Q.	10.0	9.5	9.8	10.3	9.8	10.0
Average				10.1			10.3

100 cc. of serum before treatment, 10 mgm. after six weeks and 9.9 mgm. after three months (see table 1). The group getting calcium and thyroid for three months averaged 10.1 mgm. before treatment and 10.3 mgm.

after it (see table 2). The group receiving calcium alone for three months averaged 10.5 mgm. before and 10.5 mgm. after treatment (see table 3). That group taking thyroid alone for three months averaged 10.4 mgm. before and 10.5 mgm. after treatment (see table 4). The

TABLE 3

Group 3. Calcium for 2½ months

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		BEFORE TAKING			AFTER 2½ MONTHS		
		Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum		
		October 20	October 30	Average	January 7	January 11	Average
A. J.	F. A. A.	10.3	10.8	10.6	10.3	11.1	10.7
P. B.	F. A. B.	10.3	10.5	10.7	10.2	9.3	9.8
M. F.	F. A. B.	10.3	9.1	9.7	10.6	9.8	10.2
S. K.	F. A. A.	10.1	9.8	10.0	10.4	10.4	10.4
M. A.	F. A. Q.	11.5	10.3	10.9	10.8	10.3	10.5
L. D.	F. A. A.	11.0	10.8	10.9	10.5	10.8	10.7
J. P.	F. A. B.	10.4	9.6	10.0	10.6	10.0	10.3
W. L.	F. A. A.	11.2	10.8	11.0	11.2	11.1	11.1
Average				10.5			10.5

TABLE 4

Group 4. Thyroid for 2½ months

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		BEFORE TAKING			AFTER 2½ MONTHS		
		Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum		
		October 18	November 2	Average	January 12	January 15	Average
J. F.	F. A. A.	10.3	10.6	10.4	Left institution		
J. W.	M. A. Q.	10.2	10.6	10.4	10.8	10.2	10.5
V. M.	F. A. Q. (Fibroid)	10.4	10.3	10.3	10.8	10.8	10.8
F. L.	F. A. Q. (Fibroid)	10.6	9.8	10.2	11.0	10.2	10.6
J. B.	F. A. A.	11.3	10.8	11.0	10.5	11.5	11.0
J. M.	F. A. A.	10.9	9.9	10.4	10.8	10.3	10.6
M. M.	F. A. B.	10.9	Left institution				
M. R.	F. A. B.	10.8	10.4	10.6	10.8	10.1	10.5
A. K.	F. A. A.	9.8	9.9	9.9	9.6	9.6	9.6
Average				10.4			10.5

control group averaged 10.3 mgm. at the beginning and 10.8 mgm. three months later (see table 5). The markedly active toxic group that received calcium and thyroid for three months averaged 7.7 mgm. before and 7.8 mgm. after treatment (see table 6).

TABLE 5

Group 5. Controls

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		AT START			AFTER THREE MONTHS		
		Milligrams calcium per 100 cc. serum			Milligrams calcium per 100 cc. serum		
		September 24	October 14	Average	January 9	January 15	Average
F. R.	F. A. C.	10.0	10.5	10.3	10.4	10.8	10.6
R. D.	F. A. B.	11.1	10.3	10.7	10.9	10.8	10.9
A. A.	F. A. B.	9.4	8.8	9.1	Left institution		
E. O'N	F. A. B.	10.2	11.0	10.6	11.1	11.3	11.2
T. A.	F. A. B.	10.1	10.5	10.3	Left institution		
J. M.	F. A. A.	10.9	10.3	10.6	Left institution		
W. McC.	F. A. A.	10.3	11.0	10.6	10.8	10.7	10.8
J. E.	F. A. B.	11.2	10.8	11.0	11.3	11.1	11.2
J. O'N.	F. A. A.	10.2	11.1	10.6	10.1	10.8	10.4
J. C.	F. A. B.	9.6	9.6	9.6	10.8	10.3	10.5
Average				10.3			10.8

TABLE 6

Group 6. Calcium and thyroid for three months; markedly active toxic group

PATIENT'S INITIALS AND N. T. A. CLASSIFICATION		BEFORE TAKING	AFTER THREE MONTHS
		Milligrams calcium per 100 cc. serum	Milligrams calcium per 100 cc. serum
J. C.	F. A. C.	7.6	7.7
P. L.	F. A. C.	7.4	7.2
J. M.	F. A. C.	8.1	8.4
J. O.	F. A. C.	8.2	7.9
E. P.	F. A. C.	7.7	8.1
J. S.	F. A. C.	7.6	7.9
A. V.	F. A. C.	7.3	7.4
F. R.	F. A. C.	8.2	8.0
A. C.	F. A. C.	8.0	8.2
W. J.	F. A. C.	7.2	7.6
Average		7.7	7.8

DISCUSSION

These results show practically no change in the serum-calcium figures in groups 1 to 5 inclusive, before or after treatment. In fact, the findings in all five groups very closely approximate one another, and are all well within normal limits.

Group 6, the markedly active toxic group, showed a marked drop

below the normal serum-calcium figure (normal average 9.6 to 10.8 mgm.). There was an average decrease of 25 per cent in this group. This apparently indicates that there is a decided loss of calcium from the blood in the terminal stages of pulmonary tuberculosis.

REFERENCES

(1) MUGGIA: Riforma Med., 1921, xxxvii, 1050.
(2) MASON: J. Biol. Chem., 1921, xlvii, 3.
(3) DENIS AND MINOT: Ibid., 1920, xli, 357.
(4) HOWLAND AND MARRIOTT: Quart. J. Med., 1918, xi, 289.
(5) JACOBOWITZ: Jahrb. f. Kinderhk., 1920, xcii, 25.
(6) MATZ: Amer. Rev. Tuberc., 1925, xi, 250.
(7) MAVER AND WELLS: Ibid., 1923, vii, 5.
(8) NOVAK AND HOLLENDER: J. Am. Med. Assn., 1923, lxxxi, 2003.
(9) KRAMER AND TISDALL: Bull. Johns Hopkins Hosp., 1921, xxxii, 44.

THE IMPORTANCE OF THE APICAL ROENTGENOGRAM IN PULMONARY TUBERCULOSIS[1]

FELIX BAUM AND LOUISA T. BLACK

The difficulty of the early X-ray diagnosis in apical pulmonary tuberculosis is well known. The general plate in many cases does not show the details in the apical region, due to the fact that the clavicle obstructs the view by covering parts of one or more intercostal interspaces.

Numerous attempts have been made to make a clear apical picture. Assmann, for instance, publishes in his book, *Klinische Roentgendiagnose innerer Erkrankungen, 2d Auflage*, the method of Albers-Schoenberg, which he modified as follows: The thorax is resting on a triangular pillow with the head of the patient hanging over the edge, the middle of the tube being focused on the jugulum (fig. 1). In this way, he claims, he is able to show the first interspaces as large as possible by projecting the disturbing clavicular shadows downward.

We have tried this method several times, and have come to the conclusion that the interspaces are not seen as clearly nor do they appear as large as when the rays are directed from the opposite side.

More recently, according to this conclusion, we tried the following technique (fig. 2):

The patient is placed on a horizontal radiographic table and rests against a wooden pillow, which is built at an angle of 50°. On the incline is placed a feather pillow, which prevents the casette from slipping. This makes the incline about 60°. The patient is instructed to press lightly on the table with the elbows or hands, so as to raise the ends of the clavicle. The X-ray tube is placed at an angle of 20° and is focused on the sternum where the latter articulates with the clavicles. All apical examinations are made on 8 x 10 duplitized films with doubly intensifying screens, 88 kv., 60 ma., and at a distance of 29 inches, the time of exposure being governed by the size of the patient (usually $\frac{1}{4}$ of a second).

As the chests of all patients are not of the same shape, it is sometimes necessary to change the angle of the tube so as not to project the hila into the apices.

[1] From the National Jewish Hospital for Consumptives, Denver, Colorado.

The advantages of this method are (1) the clavicle is projected above the first rib and does not obstruct the view and (2) the first, second and third interspaces can be seen in their entirety.

The first film (fig. 3) shows a normal apical picture, the Y-like lines representing normal pulmonary vessels.

Figure 4 reveals beginning tuberculosis in both apices, the right more than the left, with peribronchial thickening throughout the three upper interspaces.

The importance of the apical plates is most evident in the following two cases in which thoracoplasty was recommended and active tuberculosis in the so called good lung had to be excluded.

Figure 5: General plate reveals a large cavity in the left apex, with extensive tuberculous involvement in the lower part of the left lung. The right lung shows no signs of activity in the apex, whereas in the apical plate (fig. 6) definite peribronchial thickening in the second and third interspaces is evident.

Figure 7: Numerous cavities and tuberculous involvements throughout the whole right lung. Questionable findings in the left apex. Figure 8 reveals in the apical plate definite tuberculous infiltration in the left second interspace (see arrow).

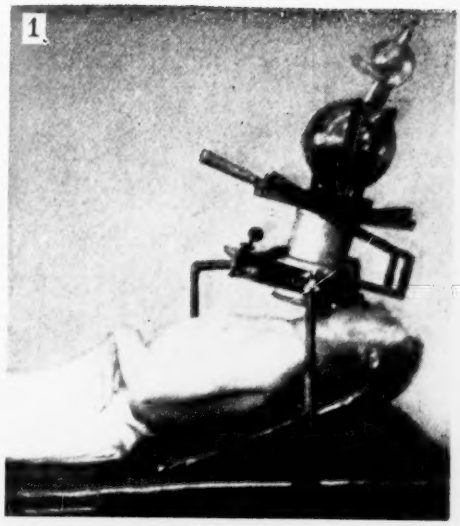
The following plates are made from patients with no physical signs and with mild subfebrile temperatures for several weeks up to 99.2°F. The general plates (figs. 9 and 11) are without definite pathological findings. The corresponding apical plates (figs. 10 and 12), however, show tuberculous nodules in the left second and third interspaces.

SUMMARY AND CONCLUSION

The apical plate is of greatest importance in certain cases of pulmonary tuberculosis. Its value seems to lie in cases of questionable apicitis, as well as in artificial-pneumothorax or thoracoplasty cases in which tuberculosis in the so called good lung must be excluded.

DESCRIPTION OF FIGURES

FIG. 1. ALBERS-SCHOENBERG METHOD OF APICAL PICTURE AS MODIFIED BY ASSMANN
FIG. 2. OUR TECHNIQUE OF THE APICAL ROENTGENOGRAM
FIG. 3. NORMAL APICAL PLATE, THE Y-LIKE LINES REPRESENTING NORMAL PULMONARY VESSELS
FIG. 4. BEGINNING TUBERCULOSIS IN BOTH APICES, THE RIGHT MORE THAN THE LEFT, WITH PERIBRONCHIAL THICKENING THROUGHOUT THE THREE UPPER INTERSPACES
FIG. 5. GENERAL PLATE: LARGE CAVITY IN THE LEFT APEX, EXTENSIVE TUBERCULOUS INVOLVEMENTS IN THE LOWER PART OF THE LEFT LUNG; RIGHT LUNG SHOWS NO SIGNS OF ACTIVITY IN THE APEX
FIG. 6. CORRESPONDING APICAL PLATE TO FIG. 5. THIS SHOWS THE SAME CAVITY IN THE LEFT APEX AND DEFINITE PERIBRONCHIAL THICKENING IN THE 2ND AND 3RD INTERSPACES ON THE RIGHT
FIG. 7. GENERAL PLATE: NUMEROUS CAVITIES AND TUBERCULOUS INVOLVEMENTS THROUGHOUT THE RIGHT LUNG. QUESTIONABLE FINDINGS IN THE LEFT APEX
FIG. 8. CORRESPONDING APICAL PLATE TO FIG. 7. RIGHT SIDE AS IN GENERAL PLATE. LEFT SIDE SHOWS A DEFINITE TUBERCULOUS INFILTRATION IN THE 2ND INTERSPACE (SEE ARROW)
FIG. 9. GENERAL PLATE WITHOUT PATHOLOGICAL FINDINGS IN THE APICES
FIG. 10. CORRESPONDING APICAL PLATE TO FIG. 9. MARKED PERIBRONCHIAL LINES WITH TUBERCULOUS NODULES IN THE LEFT 2ND AND 3RD INTERSPACES
FIG. 11. GENERAL PLATE WITHOUT PATHOLOGICAL SIGNS IN THE APICES
FIG. 12. CORRESPONDING APICAL PLATE TO FIG. 11. TUBERCULOUS PERIBRONCHITIS IN THE 2ND AND 3RD LEFT INTERSPACES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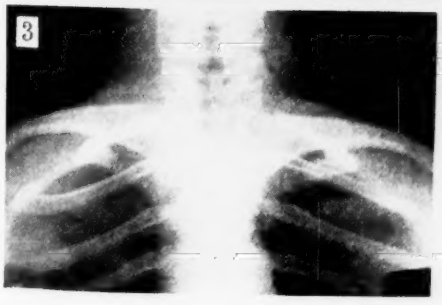
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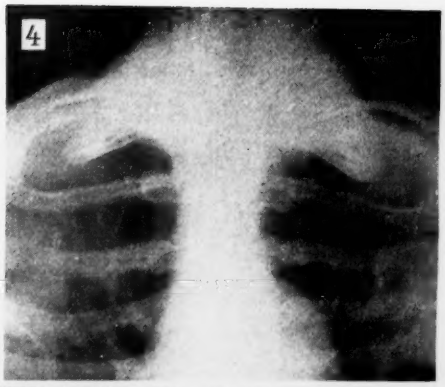
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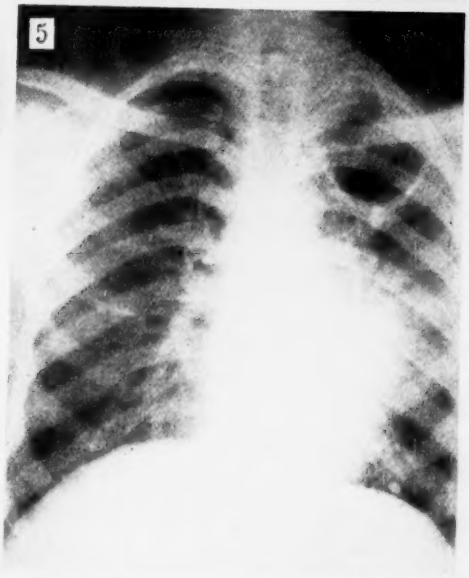
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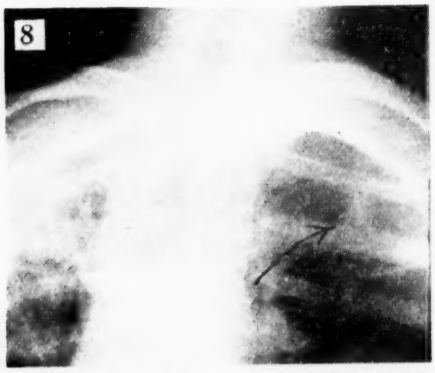
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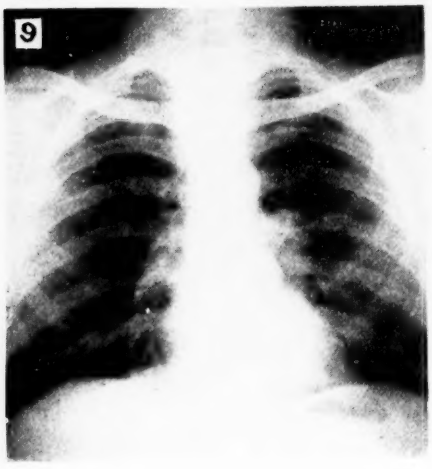
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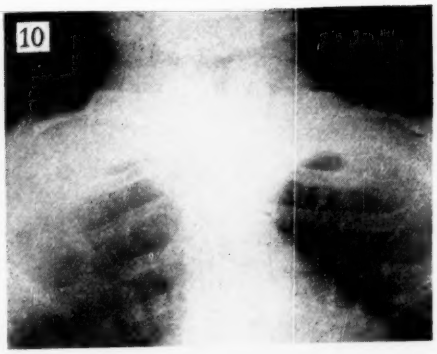
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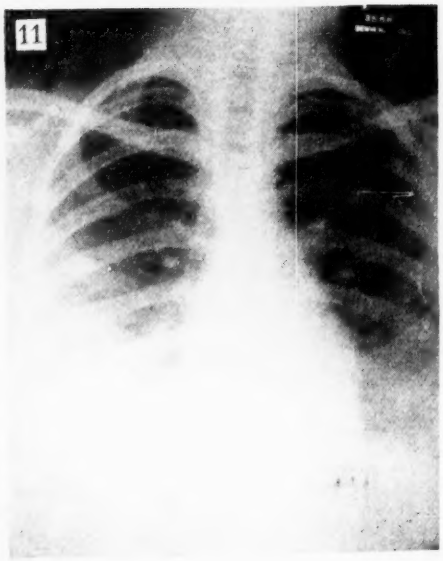
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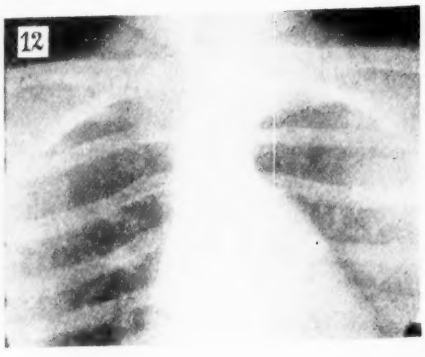
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COMPLEMENT FIXATION IN TUBERCULOSIS

IV. STUDIES ON THE NATURE OF THE ANTIBODY[1]

MAX PINNER

This study was undertaken in order to determine with which portion of the serum the complement-fixing antibodies in tuberculosis are associated or, maybe, identical. Without going into all the detail of the literature concerning this question it may be stated that the two chief opinions regarding this problem are as follows:

1. These antibodies are closely associated with the globulin fraction of the serum (Nishida and Petroff (1)).
2. These antibodies are closely related with the lipoidal substances of the serum (Hodge and MacLennan (2)).

Nishida and Petroff base their opinion on the fact that sera, the globulin fraction of which has been removed by half-saturation with ammonium sulphate, lose their complement-fixing qualities, and that the substances which can be extracted from sera by means of petrolic ether, carbon disulphide, carbon tetrachloride, acetone, ethyl alcohol, methyl alcohol, ether and benzole, suspended in saline solution, do not deviate complement. The authors state therefore, "that the globulins of the sensitized sera contain the antibodies which give rise to the complement-fixing phenomenon." They found the same thing to be true with the serum of patients giving a positive fixation. But their final conclusion is rather guarded: "They (the complement-fixing antibodies) are not lipins chemically, but are either globulins or are absorbed by the globulin."

But in precipitating the globulins of sera with salts or acids, the lipoids are precipitated with the globulins and are found in the precipitate and not in the filtrate.

Example: Serum 872 contains 150 mgm. cholesterol per 100 cc. This serum is precipitated with ammonium sulphate (full saturation) and filtered. The filter residue contains again 150 mgm. cholesterol per 100 cc. of serum. The filtrate does not contain any cholesterol.

[1] From the Laboratory of the Municipal Tuberculosis Sanitarium, Chicago, Illinois, and the Department of Pathology, College of Medicine, University of Illinois.

The same findings were obtained, when only the globulins were removed by half-saturation. The cholesterol findings were taken as indication for the whole lipoidal group, which seems to be justified considering their similar solubility, but it is a certainty that the removal of the serum proteins, by means of salt precipitation, removes at least the cholesterol also. It appears therefore, possible, that Nishida and Petroff's findings may be explained by the fact that their globulin-free serum was free from lipoids as well.

Hodge and MacLennan arrived at their conclusions because they found that sera extracted with alcohol, ether and chloroform lost their complement-fixing power, and that sera whose euglobulin fraction was removed by saturation with CO_2 retained these antibodies. But these authors were not able to demonstrate the presence of the antibodies in the extracted fraction of the serum. This may be due to the fact that these extracts were found to be highly anticomplementary. The results obtained by these authors may indicate either of two things: first, that the antibodies have been extracted by fat solvents and are therefore of a lipoidal nature, or that the action of ether destroyed the antibodies. This latter possibility will be discussed in a later part of this paper.

Since it is a well established fact, proved again by Nishida and Petroff, that antibodies do not dialyze, the crystalloids of the serum were entirely excluded from this discussion and it remained to determine whether the antibodies really follow the globulins or the cholesterol.

Example: Equal amounts of Serum B, which gives a 4-plus complement fixation, are precipitated by full saturation with ammonium sulphate and filtered. The filtrate is dialyzed against running water until free from SO_4 ions, and then rendered isotonic with NaCl. This filtrate is negative in the complement-fixation test. Both residues are dried in the incubator over night. The one is dissolved in distilled water, dialyzed until no SO_4 ions are present, made isotonic, and tested for complement-fixing antibodies; this fraction gives a 4-plus reaction. The other residue is extracted with dry ether in a Soxhlet for one hour, dried and submitted to the same treatment as the other residue. This fraction gives a negative reaction with three different antigens.

Sera with negative complement fixations were submitted to the same treatment; but none of the fractions ever gave a positive reaction. This experiment proved that the antibodies are always found in the fraction containing the cholesterol, and it gave encouragement to attempt a more conclusive proof for the predominating importance of the lipoids in complement fixation; the aim being to obtain either a lipoid-free or a protein-

free serum fraction, in which the antibodies could be demonstrated by complement fixation.

In the experiments mentioned above, it was shown that precipitation with ammonium sulphate does not separate the proteins from the lipoids; the same is true for precipitation with trichloracetic acid or with sodium tungstate and sulphuric acid.

In analogy with the findings of Hodge and MacLennan it was easy to demonstrate that positive sera extracted with ether lose their complement-fixing power. The method employed in these experiments was the one advocated by Kolmer (3) in his studies on nonspecific complement fixation. But these findings again may mean either that the antibodies are soluble in ether or are destroyed by ether.

Then it was tried to convert negative sera into positive ones by the addition of different amounts of cholesterol, cholesterol-ester and lecithin. The amounts added were so chosen as to bring the lipoid content up to the content found in strongly positive sera. The results were negative, the sera either giving a negative complement-fixation test, or almost always becoming highly anticomplementary. In another attempt negative sera were extracted in the shaking machine with ether; alcohol was not used, so as not to precipitate any of the proteins. To these extracted sera were added saline suspensions of the whole lipoids previously extracted by ether and alcohol from strongly positive sera. Again these preparations proved to be either negative or anticomplementary. In another series of experiments it was tried to demonstrate the antibodies in the lipoids extracted from positive sera. Saline suspensions of lipoids extracted with ether, or ether and alcohol, from positive sera were tested; these suspensions proved to be highly anticomplementary. Hodge and MacLennan reported the same occurrence with their lipoidal extracts. When these lipoidal suspensions, however, were injected into rabbits, whose serum gave a negative test before the injection, in some instances the serum of these rabbits would give a weak complement deviation three and twenty-four hours after the injection. These findings suggested that with lipoids from positive sera it may be possible to transfer antibodies. But these results were not uniform enough to be convincing; the reported findings were obtained only in about 30 per cent of the animals treated; and furthermore it was found that normal rabbit serum not infrequently gave a positive complement fixation. Kolmer and Trist (4) report that inactivated rabbit sera gave

nonspecific complement fixation with lipoidal antigens in 33 to 49 per cent of the sera. All the antigens used in this series of experiments contained lipoids.

At last, attempts were made to obtain a protein-free serum fraction by digesting the serum proteins with pepsin and hydrochloric acid or with trypsin, and removing the split products by dialysis. After many failures the following technique was found to be satisfactory: The sera are heated in a water-bath at 60°–65° for $\frac{1}{4}$ hour; this coagulates the serum to a soft gelatinous consistence. This heating process was found necessary, in order to destroy the antitryptic power of the serum. Four

TABLE 1

Sera and their antibody content before and after digestion

ORIGINAL SERUM	STRENGTH OF REACTION		CLINICAL DIAGNOSIS
	Before digestion	After digestion	
11	++++	+++	F. A. B.
12	++++	+++	Incipient B.
G	++++	+++	M. A. B.
H	++++	+++	F. A. B.
S	++++	+++	M. A. A.
7	++++	++	Incipient B.
8	+	+	Incipient A.
5	++	++	M. A. A.
9	++++	++	M. A. A.
16	++++	+++	M. A. A.
H	++++	++	F. A. B.
21	++++	+++	M. A. B.
17	++++	+	F. A. B.
Pooled serum	++++	+++	Active pulmonary tuberculosis
A	−	−	Cord blood from normal infants
D	−	−	Cord blood from normal infants

or nine times the amount of saline solution is added to the serum, shaken and stirred until all the coagulated serum is dissolved or at least suspended in the saline solution. This mixture is transferred into a dialysis bag. As dialysis bags, collodion bags or fish-skin condoms were used. A small amount of trypsin is added and mixed with the liquid. The bags are immersed in water in a large container, and the whole is placed in an incubator. The water is changed three times in 24 hours. After three to five days the content of the bags is protein-free according to the chemical tests (1) boiling under addition of acetic acid and (2) sulphosalicylic acid test; and it does not contain any noncoagulable proteins or lower split products which could be detected by the biuret test or by the

ninhydrin reaction. This deproteinized serum is then made isotonic by the addition of 10 per cent NaCl and its anticomplementary unit is determined. Half of the anticomplementary unit is taken for the test. In the beginning of this work a few sera still gave a positive biuret and ninhydrin test, but did not contain any heat-coagulable proteins after the treatment stated above; the antibodies were retained. It is not feasible to cover the sera with toluol during the process of deproteinizing, because sera treated this way lost their antibodies, very likely because some of the lipoids were taken up in the toluol.

It is difficult to make a quantitative comparison between the titre of the original serum and the digested product, because during the process of digestion and dialysis the volume of the diluted serum changes very considerably. But for our problem it was sufficient to know that complement-fixing antibodies are found in the protein-free fraction. It was found, however, that half the anticomplementary unit of the final product corresponds approximately to half the amount of the original serum and that it gives a test of a slightly decreased strength.

COMPLEMENT-FIXING ANTIBODIES AND SERUM LIPASES

Since it is well known that the tubercle-bacillus lipoids play the most important, if not the exclusive rôle in the antigens for complement fixation, interest in a possible relation between serum lipases and antibodies has been aroused anew. Levinson and Petersen (5) showed in their examinations that the serum lipases, determined by the ethyl-butyrate method, do not at all parallel the complement-fixing antibodies. That such a parallelism does not exist is indirectly indicated by the following experiment:

Sera, which were strongly positive in the complement-fixation test, were treated with quinine sulphate in order to paralyze the action of the serum lipase which, according to Rona (6), is destroyed by the action of quinine. Two hours later the sera were again tested for complement-fixing antibodies; in no case was a decrease of complement-deviating strength observed. It can, therefore, be considered as proved that the ethyl-butyrate lipases are not identical with the complement-fixing antibodies.

But Much (7) writes, "The search for blood lipases or lymphocytes or organ lipases with the unspecific tributyrate, etc., is a wrong way and unsuccessful. If one wishes to seek the specific lipases (and that is what one wants) one has, of course, to look for them by means of the specific

substances. One has, therefore, to use not a random lipoid, but the lipoids of the tubercle bacillus itself." And Wells (8) says, "The literature on this subject is very confused because so many authors have failed to recognize that the capacity of a fluid to cause cleavage of a simple ester, like ethyl butyrate (esterase), may have no relation to its capacity to split natural fats (*lipase*)."

Tubercle-bacillus lipoids were prepared and some of the extracts which proved most active in complement fixation (9) were evaporated to dryness in the water-bath. The residues were brought into distilled water, and vigorously shaken for a long time, and finally filtered through filter paper. The resulting filtrates were more or less opaque fluids, and contained some of the lipoidal substances in fine suspension, as proved by evaporating and by stalagmometric determinations. They did not contain any proteins or protein cleavage products, tested by boiling test, sulphosalicylic acid, and biuret and ninhydrin tests. Similar emulsions were prepared with the ether and acetone-soluble lipoids. These emulsions were mixed with tuberculous and with normal sera in the presence of a buffer solution (pH 7.4), and the surface tension was determined by means of the stalagmometer immediately after mixing and at various intervals, 2, 5, and 24 hours thereafter. In no case was an increase in the number of drops observed which would indicate a lipolytic action of the serum on the tubercle-bacillus lipoids.

Another procedure was chosen. The lipoids, after evaporation of their solvents, were spread in a thin and even layer in Petri dishes and drops of serum were placed upon this layer. This arrangement was suggested by Bergel's (10) procedure employed in the demonstration of the lipolytic action of lymphocytes. In this series of experiments it was not possible to demonstrate any lytic action of the tuberculous serum on tubercle-bacillus lipoids.

INFLUENCE OF STANDING ON TUBERCULOUS ANTIBODIES

Wedel (11) reports that a considerable percentage of tuberculous sera which, tested shortly after the withdrawal of the blood, gave a negative complement-fixation test, will react positively when tested again, after standing for a week or longer. It was of interest to repeat this procedure, with the idea of ascertaining whether only tuberculous sera behave in this way or whether nontuberculous sera, also, will react more frequently with tubercle-bacillus antigens after standing for a certain

length of time. Negative sera obtained from active tuberculosis patients, from children with lymph-node tuberculosis and from nontuberculous patients were examined. The latter group comprised a large percentage of bloods taken from the umbilical cords of healthy infants with nontuberculous mothers. The interval between first and second test varied between 5 and 28 days. The sera were kept in the icechest, and were retested only when they were not contaminated. Table 2 shows the results in the three different groups of patients.

Although the first and second groups show by far the highest percentage of positive reactions in originally negative sera, the same occurrence is found in undoubtedly nontuberculous sera, as in the sera from the healthy newborn of nontuberculous mothers.

TABLE 2

Incidence of negative sera becoming positive after standing 5 to 28 days in the icechest

	ACTIVE PULMONARY TUBERCULOSIS	LYMPH-NODE TUBERCULOSIS	NON-TUBERCULOUS
Number of sera	105	31	96
Positive in second test	51	11	18
Percentage of positives in second test	48.6	35.5	18.8

SENSITIVENESS OF ANTIBODIES TO ETHER

If a small amount of ether is added to fresh Wassermann-positive sera, and these sera are afterward inactivated, they will frequently give a negative complement fixation (Forssmann (12)). For the studies reported here it was of interest to know whether similar findings could be obtained with tuberculous sera. A series of fresh, tuberculous, 4-plus sera was treated in the manner stated above, and it was found that about 50 per cent of the sera examined were negative after the ether treatment, that some remained positive, and that the rest became anticomplementary. These results must be considered in any experiments in which ether extraction is employed. The disappearance of antibodies after ether extraction may always be due either to true extraction or to destruction by the action of ether.

DISCUSSION

It has been shown in these studies that the conflicting results of Nishida and Petroff and of Hodge and MacLennan are due to the differ-

ent technique that has been used by these authors. Either technique does not permit of a definite conclusion; the one employed by Petroff does not separate the lipoids from the globulins, and the method used by Hodge and MacLennan leaves it undecided whether antibodies were truly extracted or destroyed by ether. The method used here produces sera which are chemically protein-free; whether they might contain minute traces of proteins which cannot be detected by our usual chemical tests, and which are not split by the action of trypsin (proteins having the linkage between the CO and the NH group are not digested by trypsin), cannot be stated with certainty. Biological methods of protein determinations may help to decide this question. But the work of the last decade has made it very questionable whether hypersensitiveness and precipitins can be produced only by protein substances. The solubility of the substance in question could not be determined, and the fact that ether extraction removes them may be due either to their ether solubility or to their ether sensitiveness.

Antibodies in chemically protein-free serum fractions have been demonstrated by Huntoon (13) in antipneumococcic sera. This author also employed trypsin digestion, but the split products of proteins have apparently not been removed by dialysis. Stuber (14) reported that agglutinating antibodies could be extracted from antityphoid sera by fat solvents; but Krumwiede and Noble (15) were not able to duplicate his results.

As most of the studies on antibodies have been done by methods of extraction or precipitation a reëxamination with the method described here may lead in some cases to different results. Work of this nature on different antisera is in progress in our laboratory.

The fact that specific lipases could not be demonstrated by the two methods employed in this work does not definitely prove that they are not present in tuberculous sera. The possibility is suggested that the lipolytic action of the tuberculous organism is entirely a cellular phenomenon.

With all the different tubercle-bacillus antigens used in these studies (9) nonspecific fixations may occur. Wedel's observation that some negative sera from tuberculosis patients deviate complement after standing for a week is not limited to sera from tuberculosis patients. It must be stated, however, that our examinations were not made with the same antigen as in Wedel's work.

SUMMARY

1. Sera of tuberculosis patients with positive complement fixation were digested with trypsin and dialyzed simultaneously until chemically free from proteins and protein split-products; these preparations still contained complement-fixing antibodies.

2. The complement-fixing tuberculous antibodies are therefore not of the nature of globulins. They are either lipoids or proteins which are not broken up by the action of trypsin, that is, proteins with the CO-NH linkage.

3. The results of previous investigators, which are at variance with the results presented here, are explained by the technique of their experiments.

4. In accordance with previous investigators, it has been demonstrated that the complement-fixing antibodies have apparently no quantitative or qualitative relation to the ethylbutyrases ("lipases"). True lipases with a specific action on tubercle-bacillus lipoids could not be detected in sera with complement-fixing qualities.

5. The phenomenon, that sera of tuberculosis patients, which 24 hours after the withdrawal of the blood give a negative complement-fixation test, become positive after standing for a week or more (Wedel), is not specific for tuberculous sera; the same occurrence is observed, although in a much smaller percentage, in sera of undoubtedly nontuberculous persons.

6. Complement-fixing antibodies may readily be destroyed by ether. Any procedure which uses ether extraction is therefore to be excluded from examinations for complement-fixing antibodies.

REFERENCES

(1) Nishida and Petroff: Amer. Rev. Tuberc., 1920, iv, 322.
(2) Hodge and MacLennan: J. Immunol., 1922, vii, 253.
(3) Kolmer: J. Infec. Dis., 1916, x, 46.
(4) Kolmer and Trist: Ibid., 1916, x, 20.
(5) Levinson and Petersen: Amer. Rev. Tuberc., 1923, vii, 278.
(6) Rona, Petow and Schreiber: Klin. Wchnschr., 1922, i, 2366.
(7) Much: Handb. d. Tuberc., 3 Aufl., Bd. i, 1923.
(8) Wells: Wells, DeWitt and Long, The chemistry of tuberculosis, 1923.
(9) Pinner: Amer. Rev. Tuberc., 1925, xii, 142.
(10) Bergel: Die Lymphocytose, Berlin, 1921.
(11) Wedel: J. Immunol., 1918, iii, 351.
(12) Forssmann: Biochem. Zeitschr., 1922, cxxx, 154. Klin. Wchnschr., 1922, i, 1054.
(13) Huntoon: J. Immunol., 1921, vi, 117.
(14) Stuber: Münch. med. Wchnschr., 1915, lxii, 1173. Biochem. Zeitschr., 1916, lxxvii, 273.
(15) Krumwiede and Noble, J. Immunol., 1921, vi, 211.

THE ATTENUATION OF TUBERCLE BACILLI IN INCUBATED TUBERCULOUS TISSUES

Further Observations[1]

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Since 1916 we have been studying the effects of transplanted tuberculous tissues in the guinea pig. Our work has been in part a review, with modifications, of the earlier studies of Manfredi and Frisco (1) and Bartel and his associates (2), (3), (4), but it has also led into a number of related lines of investigation. The present paper comprises a brief review of results previously reported (5), (6), (7), (8), (9), (10), (14), and an account of work done since our last communication.

REVIEW OF PREVIOUS WORK

The main points noted heretofore are as follows:

1. The disease produced in the guinea pig, by planting under the skin fresh tuberculous lymph nodes and pieces of tuberculous liver and spleen from other guinea pigs, has not differed in any constant or striking way from the disease produced by inoculation with fairly large doses of free tubercle bacilli from virulent cultures. In some instances, however, the planting of fresh tuberculous lymph nodes results in a relatively chronic disease.

2. When we repeated this procedure many times, planting nodes from the first series of pigs in a second series, and so on, there was no obvious increase or decrease in the severity of the disease produced, or in the virulence of the cultures recovered.

3. If the tuberculous tissues were incubated in test-tubes of sterile normal salt solution at 37°, they showed a progressive loss of virulence when planted in fresh guinea pigs. Most of these experiments have been done with lymph nodes, a few with pieces of liver and spleen. The rate of attenuation seems to be variable, but, in general, tissues incubated from 2 to 8 days produce a chronic disease lasting from 6 months to 2 years, while tissues incubated 10 days or longer produce either chronic disease or no disease at all.

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4. Loss of virulence does not occur at anything like this rate if the tissues are kept in the icebox.

5. If salt solution emulsions of free virulent tubercle bacilli are kept anaerobically at 37°, they become attenuated in about the same time as the bacilli in tissues, but this is not the case with anaerobic preparations kept in the icebox, nor with aerobic emulsions kept in the incubator. Whether the loss of virulence in incubated tissues is actually due to deprivation of oxygen, or to products of tissue lysis is not certain. The work of Corper, Lurie and Kretschmer (12) emphasizes the importance of the latter possibility. On the other hand, experiments of Boissevain in this laboratory seem to indicate that bactericidal products of lysis may not develop if the tissues remain absolutely sterile. Loss of virulence does occur in incubated tissues in which no contamination can be demonstrated. Fatty acids are among the reported products of tissue autolysis, and some of these have been shown by McJunkin (13) and by Boissevain to be bactericidal for the tubercle bacillus.

6. The chronic disease produced by incubated tissues has some peculiar features besides its duration. The usual ulceration of the skin overlying the planted tissue may not occur. If an ulcer forms, it may heal and remain healed until death, or it may heal, reopen, and heal, and even repeat this cycle several times; that is, these ulcers of primary inoculation often behave like the typical ulcer of second inoculation in "Koch's phenomenon." The generalized lesions usually show very marked fibrosis. This occurs in the liver, and especially in the lymph nodes all over the body. Palpable nodes, which have enlarged greatly in the early months of the disease, are sometimes reduced to their normal size by the time death occurs, though more commonly they are found to be moderately enlarged and very densely fibrosed, but still showing tubercle bacilli and some evidences of activity on microscopic section.

A peculiar feature which we have noted in chronic tuberculosis of the guinea pig, produced in this way and in other ways, is paralysis and atrophy of the hind legs. Whether this is due to involvement of the cord, or of the lumbosacral nerves which lie close to the iliac nodes, is not proved, but we think the latter is the case.

Our work has been expanded and in large part confirmed by researches of Corper and his associates (11) (12).

REPORT OF RECENT EXPERIMENTS

Work completed since our last report includes observations on the following subjects:

(1) The effect of incubated tuberculous tissue plants on subsequent inoculation tuberculosis.

(2) The comparative duration of life of guinea pigs inoculated with

tissues from acute and chronic experimental tuberculosis, and with cultures recovered from acute and chronic experimental tuberculosis.

1. Seven healthy guinea pigs were given ten-day incubated tuberculous nodes, by the usual method of subcutaneous implantation. Two of them developed chronic tuberculosis. Of the other guinea pigs, which appeared to be free from disease, one was kept as a control, and when killed after 570 days showed no tuberculosis. The other four were inoculated with an emulsion of a virulent culture, 130 days after they received the nodes. All died of generalized tuberculosis, the average duration of life after inoculation being 107 days. The average life of two controls inoculated at the same time in the same way was 66 days.

2a. Eleven guinea pigs were given subcutaneous plants of bits of liver and spleen from guinea pigs which had died of the chronic experimental tuberculosis produced by incubated tuberculous tissues. All died of generalized tuberculosis, of an average duration of 112 days. There was no constant ratio between the duration of life in the original chronic case and in the animals inoculated from it.

Two controls were given similar plants from a guinea pig which had died of ordinary acute inoculation tuberculosis. Both died of generalized tuberculosis; their average duration of life was 50 days.

2b. Three guinea pigs were inoculated subcutaneously with an emulsion of a culture of tubercle bacilli recovered from a case of chronic experimental tuberculosis produced by incubated tuberculous tissue. All died of generalized tuberculosis, average duration 70 days. Two controls were inoculated with a culture from a case of ordinary acute experimental tuberculosis. Both died of generalized tuberculosis, average duration 52 days.

DISCUSSION AND CONCLUSIONS

In the case of the animals given ten-day incubated nodes, and later inoculated with virulent bacilli, the duration of life was decidedly longer than that of the controls. We may infer, as Bartel did, that there is some evidence of increased resistance as a result of this procedure. Even this is not certain, however, since the disease in the controls ran an exceptionally rapid course. The usual duration of guinea-pig tuberculosis produced by subcutaneous inoculation with moderately large doses of virulent bacilli is about three months, in our experience. In this experi-

ment the treated animals lived 3½ months and the controls a little more than two.

The life of the animals inoculated with tissues and cultures from chronic experimental tuberculosis was longer than that of controls inoculated with similar material from the acute disease. In one group it was somewhat longer than the usual experimental disease, but in the other group it was actually shorter. That is to say, the disease produced with material from chronic cases may show a tendency to chronicity, but is more acute than the original chronic disease. We have recently reported a similar observation on the Saranac Laboratory R1 strain (14).

It is of course possible that repeated implantation or injection of preparations of incubated tuberculous tissues might produce an effectively increased resistance to virulent infection. It is also possible that a strain of virulent tubercle bacilli may become so attenuated as to be absolutely incapable of producing disease. But the strongest impression which we have received, as a result of the work here reported and referred to, is that it is very dangerous to introduce into a susceptible animal any sort of material containing tubercle bacilli which are not positively known to be dead.

REFERENCES

(1) Manfredi and Frisco: Centralbl. f. Bakt., 1902–1903, Ref., xxxii, 295.
(2) Bartel and Neumann: Ibid., 1905–1906, Orig., xli, 518.
(3) Bartel and Neumann: Ibid, 1909, xlviii, 657.
(4) Bartel: Wien. klin. Wchnschr., April 19, 1906, xix.
(5) Webb, Ryder and Gilbert: Amer. Rev. Tuberc., 1918, i, 693.
(6) Webb, Ryder and Gilbert: Tr. Assn. Amer. Phys., 1918.
(7) Webb, Ryder and Gilbert: Tr. Nat. Tuberc. Assn., 1921, xvii, 288.
(8) Webb, Ryder and Gilbert: Amer. Rev. Tuberc., 1921, v, 388.
(9) Webb, Ryder and Gilbert: Tr. Nat. Tuberc. Assn., 1923, xix, 290.
(10) Webb, Boissevain and Ryder: Amer. Rev. Tuberc., 1924, ix, 534.
(11) Corper, Lurie and Kretschmer: Tr. Nat. Tuberc. Assn., 1923, xix, 285.
(12) Corper, Lurie and Kretschmer: Amer. Journ. Hyg., March, 1924, iv, 77.
(13) McJunkin: Amer. Rev. Tuberc., July, 1924, ix, 464.
(14) Webb, Ryder and Boissevain: Ibid., 1925, xi, 247.

THE INFLUENCE OF IRON ON THE GROWTH OF THE TUBERCLE BACILLUS UPON GLYCERINATED BEEF BROTH

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During an experiment intended to determine whether the presence of certain metals, in the metallic form, had any inhibitory influence on the growth of *Bacillus tuberculosis* upon glycerinated beef bouillon, it was noticed that cultures, grown on broths to which iron filings had been added, developed a heavier, more wrinkled and more tenacious pellicle than cultures grown on media without iron. The difference between the control cultures and those containing iron were so striking that a further inquiry was prompted.

Sauton (1) in 1912 reported that the addition of ammoniacal citrate of iron to a synthetic medium increased the growth of *B. tuberculosis*. Calmette (2) stated that he and Massol had previously observed the same facts. Bezançon, Philibert and Boudin, according to Frouin (4), questioned the findings of Sauton, while Borrell, deCoulon, Boez and Quimaud (3) found that small quantities of iron, in media which have a pH value of 6.9, are particularly favorable to the growth of the bacillus. Frouin and Guillaumie (4) found that the addition of the salts of iron increased the growth of the bacillus in media which contained glycerine and glucose, regardless of the reaction of the media, but that in non-glycerinated media which contained glucose the effect of the iron depended upon the reaction of the media; an increase of growth occurred in acid media (pH 5.6) and a decrease in neutral media. Frouin and Guillaumie (5) have recently reported that the increased weights of bacteria obtained from synthetic media containing iron are due to the better utilization of the glycerine.

In our laboratories there is used for the cultivation of *B. tuberculosis* a beef-infusion broth containing 1 per cent of peptone, 0.5 per cent of potassium biphosphate, and 7 per cent of glycerine by weight. The reaction of the medium is adjusted with 2N NaOH, so that 100 cc. of

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the medium requires 0.6 cc. N/1 NaOH for neutralization with phenolphthalein. This broth after titration and sterilization has a pH value of 7.1 to 7.3. Analysis of several lots of this broth shows that it contains normally from 0.02 to 0.10 mgm. of iron per 100 cc. Previous investigators have worked almost entirely with synthetic media. In some instances the opinions advanced were based upon rather meagre evidence and the conclusions reached have not been in full accord. In the present investigation an attempt has been made to determine the influence of salts of iron upon (1) the total amount and (2) the rate of growth of *B. tuberculosis* per 100 cc. of the culture fluid used in the Biochemic Division.

EXPERIMENTAL

The influence of iron upon the weight of bacilli produced upon a given volume of culture media

A number of separate experiments were carried out but, since the results in all cases were in substantial accord, it seems unnecessary to describe more than one in detail.

Experiment 1: One hundred cubic centimetres of the glycerinated broth (pH 7.3) previously described were placed in each of 24 flasks. To each of 8 of these flasks there was added 10 mgm. of ferric sulphate, to each of a second lot of 8 flasks there was added 20 mgm. of ferric sulphate, while the remaining 8 flasks served as controls, no ferric sulphate being added. All flasks[2] were autoclaved at 12 pounds pressure for 20 minutes and each was inoculated with *B. tuberculosis*, human type, strain 120. The bacteria used to seed the iron media, while of the same strain as those used to seed the controls, had passed seven generations on iron media, while the bacteria used to seed the controls had never been in contact with media to which iron had been added. The flasks were incubated for ten weeks, and the cultures were then killed by heating for three hours in an Arnold sterilizer. The pellicles on the control media were ivory white, comparatively smooth, and possessed so little cohesiveness that a very slight jar sufficed to break them. The pellicles on the iron media were tinted gray, and were very wrinkled and decidedly cohesive. The culture fluid of the iron broth became very dark brown in color, while that of the control media

[2] Unless otherwise stated, the flasks used in the experiments described in this paper were of 300 cc. capacity, and after seeding were capped with tinfoil.

was a light brown or amber color. The iron broth contained a slight precipitate, probably iron phosphate. It may be noted here that a precipitate is formed when ferric sulphate is added to the broth. After sterilization the contents of each flask were filtered separately through hardened, tared papers. The clear filtrates from each lot were separately combined and the pH values determined.[3] Each filter was washed with from 300 to 500 cc. of hot distilled water. After washing, the papers containing the bacteria were placed in separate aluminum dishes provided with covers, and dried at 100°C. until the loss in weight

TABLE 1

The influence of ferric sulphate on the growth of B. tuberculosis on glycerinated beef broth

FLASK*	WEIGHT IN GRAMS OF DRIED BACTERIA		
	From control broth	From iron broth	
		10 mgm. $Fe_2(SO_4)_3$	20 mgm. $Fe_2(SO_4)_3$
1	0.44	0.72	0.74
2	0.60	0.82	0.76
3	0.53	0.66	0.64
4	0.53	0.64	0.65
5	0.59	0.64	0.73
6	0.36	0.77	0.77
7	0.56	0.81	0.83
8	0.61	0.72	0.76
Totals.....	4.22	5.78	5.88
Average.....	0.53	0.72	0.74
Final pH.....	6.1	5.6	5.6

* The flasks of each lot were numbered consecutively from 1 to 8. The results given in vertical columns 2, 3 and 4 represent the weight of bacteria from separate flasks.

after two hours did not exceed 2 mgm. The weight of the bacteria from each flask was calculated. The results are shown in table 1.

The addition of iron to glycerinated broth resulted in a 37 per cent increase in the weight of bacteria produced.

A summary of seventeen similar experiments in which corresponding results were obtained is presented in table 2.

The results shown in table 2 agree with those given in table 1. The bacteria made more growth upon iron than upon control broth.

[3] The writer is indebted to Dr. F. W. Tilley, of the Biochemic Division, for the determination of the pH values recorded in this paper.

Although the work of others with iron citrate, as well as the favoring effect of metallic iron noted by the writer, pointed plainly to the Fe ions as the cause for the increased growth when ferric sulphate was added, it was deemed advisable to compare the action of that salt directly with that of some other sulphate. Accordingly, the following experiment was carried out:

TABLE 2

Summary of seventeen experiments concerning the influence of ferric sulphate on the growth of B. tuberculosis

EXPERIMENT	PERIOD OF GROWTH	NUMBER OF FLASKS IN EACH LOT	AVERAGE WEIGHT OF DRIED BACTERIA PER FLASK		INCREASE
			Control media	Iron media*	
	weeks		*gram*	*gram*	*per cent*
1	8	24	0.45	0.68	51
2	8	7	0.40	0.51	27
3	8	21	0.37	0.53	43
4	8	24	0.41	0.53	29
5	12	8	0.59	0.83	40
6	12	8	0.57	0.77	35
7	10	8	0.55	0.75	38
8	11	8	0.55	0.87	58
9†	13	6	0.54	0.70	29
10		8	0.53	0.75	41
11	12	4	0.55	0.96	74
12	10	4	0.62	0.88	41
13	10	3	0.68	0.85	25
14	10	6	0.63	0.80	27
15†	10	6	0.54	0.80	48
16†	8	8	0.49	0.69	40
17	11	6	0.58	0.87	44
Average per flask.....			0.53	0.75	41

* In the first four experiments from 5 to 20 mgm. of $Fe_2(SO_4)_3$ were added to each 100 cc. of media; in all others 20 mgm. was added.

† In these experiments the DT strain of *B. tuberculosis* was used; in all others the no. 120 strain was used.

Experiment 2: Twenty-four flasks of ordinary broth (pH 7.2) were divided into three lots of 8 flasks each. To one lot, which served as a control, nothing was added, to each flask of the second lot 1 cc. of ferric-sulphate solution was added, and to each flask of the third lot 1 cc. of a sodium-sulphate solution was added. The two sulphate solutions contained the same amount of SO_4 (7.2 mgm. per 100 cc.). The flasks

were seeded with human strain 120 and incubated together. The weights of bacteria were determined as described in experiment 1. The results are shown in table 3.

Although the average weight of bacteria on the sodium sulphate medium was 14 per cent greater than on the control, this difference was due to the unusually low weights obtained from two flasks, nos. 4 and 8, of the control lot. When these two flasks were not included, the average weights of bacteria from the control and sodium sulphate media were 0.61 and 0.625 gm. per flask, respectively. That is, this experiment affords no evidence that the sodium sulphate has a favorable in-

TABLE 3

The comparative influence of ferric and sodium sulphates on the growth of B. tuberculosis

FLASK	WEIGHT IN GRAMS OF DRIED BACTERIA		
	From control broth	Broth containing 7.2 mgm. SO_4 per 100 cc.	
		As Na_2SO_4	As $Fe_2(SO_4)_3$
1	0.67	0.60	0.93
2	0.57	0.60	0.55
3	0.62	0.68	0.89
4	0.47	0.68	0.95
5	0.60	0.64	0.86
6	0.65	0.56	0.95
7	0.55	0.63	0.95
8	0.31	0.62	0.95
Totals.....	4.44	5.01	7.03
Average.....	0.55	0.63	0.88
Final pH.....	5.9	5.5	6.1

fluence on the growth of the bacteria. One flask of the iron medium produced only 0.55 gm. of bacteria, but, even including that flask, the average weight of bacteria per flask on the iron medium was 0.88 gm. The results indicate that the favorable influence of the ferric sulphate is due to the Fe ions and not to the sulphate ions.

Frouin, as previously noted, stated that the addition of iron salts to a synthetic culture medium which contained glycerine and glucose increased the development of the bacteria regardless of the pH value of the media. Since the broth employed in these laboratories contains 7 per cent by weight of glycerine and small amounts of carbohydrates

derived from the beef, an attempt was made to determine the effect of the pH value of this broth upon the growth of the bacteria.

Experiment 3: Three litres of the regular glycerinated broth were brought to a reaction of approximately pH 8.4 with NaOH solution. This was then divided into six lots of 500 cc. each, and the reaction of the different lots was adjusted with N/1 HCl to the reactions shown in table 4. Each lot, in portions of 100 each, was placed in 300-cc. Erlenmeyer flasks, and to each flask 20 mgm. of ferric sulphate was added. Four flasks of each lot were seeded with the no. 120 strain of *B. tuberculosis*, and incubated for 10 weeks. The cultures were then sterilized, filtered through separate tared hardened papers, and the pH values of the combined filtrates from each lot determined. The bacteria on the papers were washed, dried and weighed. There were used as controls 4 flasks of the same broth without iron and adjusted to pH 7.3. The results are shown in table 4.

Experiment 4: Four lots of iron broth, each of which contained 20 mgm. of ferric sulphate per 100 cc., but each of which had a different pH value, were used. For each lot of the iron medium of a given pH value a control lot of the same pH value was used. In other respects this experiment was like experiment 3. The results are shown in table 5.

The results obtained in experiments 3 and 4, and summarized in tables 4 and 5, show that *B. tuberculosis* was able to grow on broth in which the original pH value ranged from 5.9 to 8+. Neither the maximum nor the minimum hydrogen-ion concentrations which would permit growth of the bacteria were determined, but the microörganism failed to grow on broth adjusted to a pH value of 4.6. In all cases, when growth took place, it was more luxuriant upon broth containing iron than upon control broth, regardless of the pH value.

From table 5 it is evident that growth upon the control medium was unfavorably affected when the initial pH value was reduced to 6.5 or less, while both tables 4 and 5 show that such increase in acidity had no similar effect upon the growth upon the medium containing ferric sulphate. Indeed, there was no evidence of retardation even when the reaction was brought to pH 5.9, but, on the contrary, both experiments suggest that, with ferric sulphate present, increase in initial acidity, within certain limits, is favorable to the development of *B. tuberculosis*.

The final pH values varied from 5.5 to 6.7. Factors which influence the final pH values of cultures of *B. tuberculosis* will form the subject of another paper.

The following experiment was carried out for the purpose of ascertaining whether other iron salts, such as the citrate or glycerophosphate were more favorable than the sulphate for the growth of *B. tuberculosis.*

Experiment 5: Twenty flasks, each of which contained 100 cc. of

TABLE 4

The influence of hydrogen-ion concentration on the growth of B. tuberculosis upon broth containing iron

FLASK	WEIGHT IN GRAMS OF DRIED BACTERIA						
	Control broth pH = 7.3	Iron broth, 20 mgm. $Fe_2(SO_4)_3$ per 100 cc.					
		pH = 8 +	pH = 7.5	pH = 7.3	pH = 7.1	pH = 6.9	pH = 6.7
1	0.617	0.914	0.820	0.887	0.940	0.917	0.990
2	0.647	0.917	0.930	0.887	0.935	0.917	0.923
3	0.695	0.932	0.967	0.912	0.896	0.905	0.891
4	0.564	0.802	0.880	0.802	0.896	0.906	0.951
Totals.....	2.523	3.565	3.597	3.488	3.667	3.645	3.755
Average.....	0.631	0.891	0.899	0.872	0.917	0.911	0.939
Final pH.....	5.5	6.5	6.2	6.5	5.7	5.7	5.7

TABLE 5

The influence of hydrogen-ion concentration on the growth of B. tuberculosis upon broth containing iron

FLASK	WEIGHT IN GRAMS OF THE DRIED BACTERIA							
	Original pH = 7.3		Original pH = 6.5		Original pH = 5.9		Original pH = 4.6	
	No iron	With iron	No iron	With iron	No iron	With iron	No iron	With iron
1	0.69	0.84	0.60	0.89	0.56	0.94	No growth	
2	0.69	0.86	0.71	0.88	0.59	0.91	No growth	
3	0.68	0.85	0.58	0.91	0.51	0.92	No growth	
Total.....	2.06	2.55	1.89	2.68	1.66	2.77		
Average....	0.68	0.85	0.63	0.89	0.55	0.92		
Final pH...	6.0	6.1	5.6	5.9	5.4	5.4		

the regular glycerinated broth, pH 7.25, were divided into four lots of 5 flasks each. One lot, to which nothing was added, served as controls. A different salt of iron was added to each of the remaining three lots. Ferric citrate was added to one, ferric glycerophosphate to another and ferric sulphate to the third. The strength of the different

iron solutions was adjusted so that 5.5 mgm. of iron were added to each flask. Each flask was inoculated with a human strain (DT) of *B. tuberculosis*. The cultures were incubated for nine weeks, then sterilized, and the weight of bacteria in each flask and the pH values of the combined filtrates from each lot determined. The results are shown in table 6.

The growth of *B. tuberculosis* was evidently influenced favorably by each of the salts of iron. If a conclusion could be based upon a single experiment it would seem that the citrate causes somewhat more luxuriant growth than either of the other two salts.

Experiment 6: In order to determine the influence of the amount of iron upon the growth of the bacteria, different amounts of ferric sulphate were added to broth, which was then seeded with the DT strain,

TABLE 6

The influence of different salts of iron on the growth of B. tuberculosis

IRON SALTS ADDED*	WEIGHT IN GRAMS OF DRIED BACTERIA						FINAL pH VALUE
	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Average	
None: control	0.54	0.56	0.56	0.49	0.56	0.54	6.3
Glycerophosphate	0.81	0.67	0.84	0.69	0.78	0.76	8.0
Citrate	0.93	1.00	0.83	0.93	0.72	0.88	6.3
Ferric sulphate	0.88	0.70	0.78	1.04	—	0.85	6.4

* The amount of iron added was in all cases the same; namely, 5.5 mgm. per 100 cc. medium.

human type, *B. tuberculosis*. After incubation for approximately two months, the cultures were sterilized and the weight of bacteria in each flask determined. The essential details of this experiment are shown in table 7.

In this experiment the concentration of iron most favorable to the growth of the microörganism was 10 to 30 mgm. of ferric sulphate per 100 cc.

The following experiment was carried out to determine whether salts of iron would exert the same favorable influence on the growth of the bacilli in a glycerinated beef broth in which the added potassium biphosphate was replaced by sodium chloride.

Experiment 7: Three lots of glycerinated beef broth were prepared. One contained one-half of one per cent of potassium biphosphate, the second contained one-half of one per cent of sodium chloride, and the

third contained one-half of one per cent of each of the two salts. One hundred cubic centimetre portions of each lot were placed in 300-cc. Erlenmeyer flasks, 20 mgm. of ferric sulphate were added to one-half of the flasks of each lot, and all of the flasks were then inoculated with strain no. 120 human type *B. tuberculosis*. After eight weeks' incubation the weight of bacteria in each flask was determined in the usual manner. The results are shown in table 8.

TABLE 7

The influence of different amounts of ferric sulphate on the growth of B. tuberculosis

AMOUNT OF FERRIC SULPHATE ADDED	WEIGHT IN GRAMS OF THE DRIED BACTERIA							FINAL pH VALUE
	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6	Average	
mgm.								
None	0.63	0.58	0.47	0.56	0.60	0.55	0.56	6.1
5	1.01	0.72	1.01	0.59	0.91	0.66	0.81	5.5
10	1.00	0.90	0.96	0.91	—	—	0.94	6.3
20	0.90	0.93	0.89	0.98	—	—	0.92	6.3
30	0.96	0.92	0.87	1.12	0.99	1.02	0.98	6.5
40	0.55	0.68	0.49	0.64	0.70		0.61	8.0
50	0.49	0.54	0.54	0.60	0.46	0.82	0.57	8.0
60	0.56	0.60	0.53	0.54	—	—	0.56	8.0

TABLE 8

The influence of potassium biphosphate and sodium chloride on the growth of B. tuberculosis upon broth containing iron

MEDIA	WEIGHT IN GRAMS OF THE DRIED BACTERIA					FINAL pH VALUE
	Flask 1	Flask 2	Flask 3	Flask 4	Average	
KH_2PO_4	0.61	0.70	0.66	0.59	0.64	6.5
$KH_2PO_4 + Fe_2(SO_4)_3$	0.89	0.91	0.86	0.83	0.87	5.9
NaCl	0.58	0.59	0.63	0.59	0.60	5.5
$NaCl + Fe_2(SO_4)_3$	0.83	0.83	0.85	0.82	0.83	5.5
$KH_2PO_4 + NaCl$	0.70	0.59	0.62	—	0.64	7.7
$KH_2PO_4 + NaCl + Fe_2(SO_4)_3$	0.80	0.67		—	0.74	6.1

Twenty milligrams of ferric sulphate per 100 cc. of broth.

The weight of bacteria from each lot of broth containing iron was greater than the weight of bacteria from the corresponding control lot. Therefore, the favorable influence of the iron was not affected by the replacement of the phosphates by sodium chloride.

THE INFLUENCE OF IRON UPON THE RATE OF GROWTH

Since it had been observed during the course of these experiments that the bacteria during the first two weeks of incubation appeared to grow more slowly upon the iron than upon the control broth, the following experiment was carried out for the purpose of ascertaining the effect of the iron upon the rate of growth rather than upon the total amount of growth.

TABLE 9

The rate of growth of B. tuberculosis on glycerinated beef broth with and without the addition of ferric sulphate

PERIOD OF INCUBATION	WEIGHT IN GRAMS OF THE DRIED BACTERIA										AVERAGE pH VALUE	
	Control broth					Ferric-sulphate broth						
	Flask 1	Flask 2	Flask 3	Average	Increase	Flask 1	Flask 2	Flask 3	Average	Increase	Control	Iron
weeks					*per cent**					*per cent**		
1	0.04	0.04	0.04	0.04		0.04	0.04	0.05	0.04		7.2	7.0
2	0.21	0.20	0.20	0.20	400	0.12	0.09	0.09	0.10	150	8.0	7.3
2.5	0.34	0.34	0.32	0.33	65	0.15	0.13	0.13	0.13	30	8.2	7.5
3	0.39	0.44	0.39	0.40	21	0.17	0.12	0.18	0.16	23	8.0	7.7
4	0.42	0.40	0.41	0.41	2	0.18	0.22	0.21	0.20	25	7.7	8.0
5	0.42	0.42	0.41	0.42	2	0.19	0.21	0.26	0.22	4	7.6	8.0
6	0.42	0.42	0.43	0.42	0	0.28	0.26		0.27	22	7.3	8.0
7	0.47	0.48	0.48	0.48	14	0.37	0.46		0.42	55	6.9	8.1
8	0.52	0.56	0.56	0.55	14	0.34	0.44		0.39	—	6.2	8.1
9	0.59	0.56		0.58	5	0.43	0.46		0.45	7	6.2	8.0
10	0.55	0.54		0.55	—	0.56	0.62		0.59	30	5.9	7.9
11	0.56	0.57		0.57	—	1.01	0.89		0.95	61	5.9	7.4
12	0.58	0.60		0.59	1	1.00	1.08		1.04	9	5.8	6.0

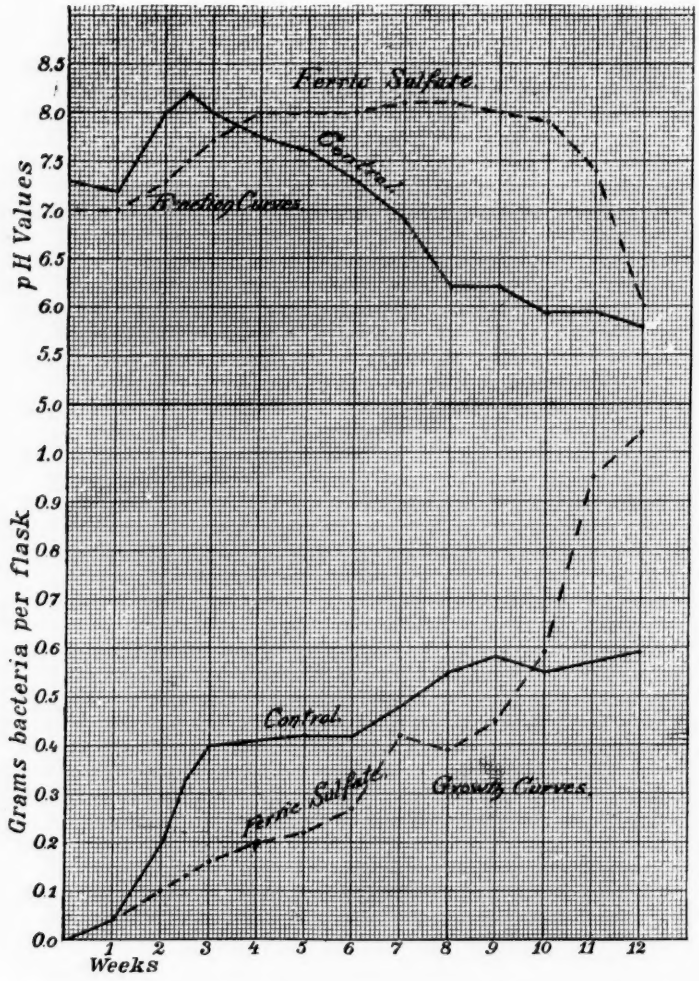
* Increase over the preceding week.

— = decrease.

Original pH values: control = 7.3; iron = 7.0.

Experiment 8: Seventy-two flasks, each of which contained 100 cc. of the regular broth, were divided into two lots of 36 flasks each. To each flask of one lot there was added 20 mgm. of ferric sulphate. The other lot served as a control. After sterilization and seeding with strain no. 120, *B. tuberculosis*, human type, the flasks were placed in three trays. In order to obtain identical conditions as far as temperature was concerned, the flasks were arranged in the trays in such a manner that the flasks of iron and control media alternated. Two

or three flasks of each lot were withdrawn at weekly intervals, sterilized and the weight of bacteria and the pH value of the culture fluid determined. The results are shown in table 9 and plotted in graph 1.

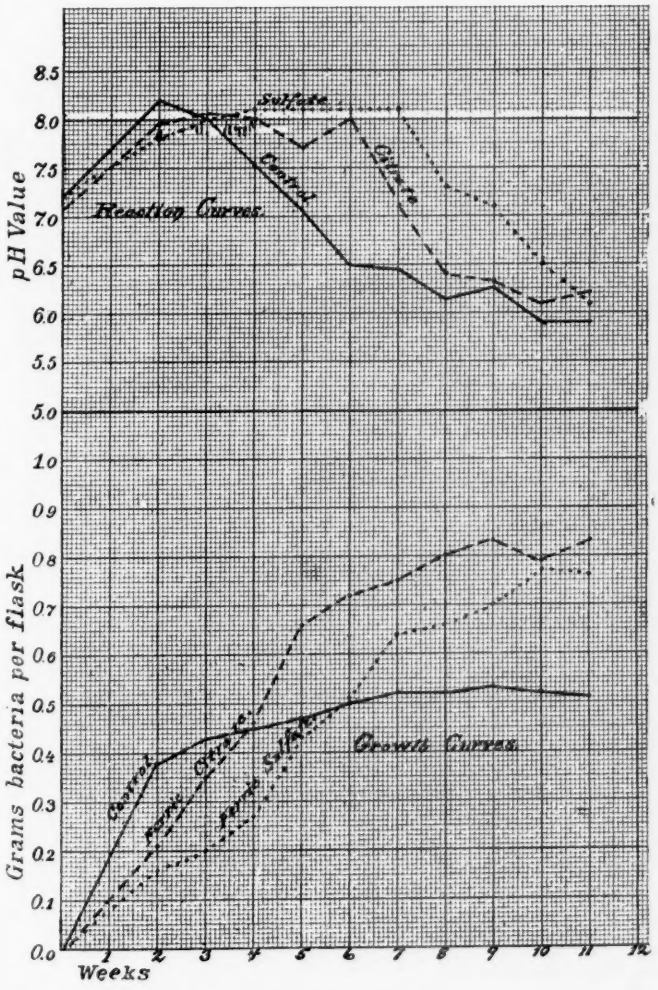


GRAPH 1. THE REACTION AND GROWTH CURVES OF B. TUBERCULOSIS UPON (1) GLYCERINATED BEEF BROTH AND (2) THAT BROTH PLUS 0.02 PER CENT OF FERRIC SULPHATE

Although at the end of twelve weeks a greater weight of bacteria was found on the iron than on the control medium, at each weighing prior to the tenth week the greater weight of bacteria was found upon the control. After the eighth week there was only a very slight increase

in the weight of the bacteria in the case of the controls, while the most rapid growth took place upon the iron medium during that time.

Experiment 9: An experiment of similar plan with the foregoing, but



GRAPH 2. THE REACTION AND GROWTH CURVES OF B. TUBERCULOSIS UPON GLYCERINATED BEEF BROTH AND UPON THAT BROTH PLUS 5.5 MGM. OF IRON PER 100 CC. IN THE FORM OF (1) FERRIC SULPHATE AND (2) FERRIC CITRATE

including in addition a broth which contained ferric citrate, was carried out. The reaction and growth curves are shown in graph 2.

In both experiments the bacteria made more total growth upon the iron than upon the control media, and in both experiments the initial

rate of growth was less on the iron than on the control. In both experiments the general form of the reaction curves upon the control media resembles that regarded by Smith as typical of the human type of microörganism, and the form of the reaction curves on the iron media conforms, for at least the greater part of the period of cultivation, to that regarded as typical of the bovine type of bacilli. It will be noted that those lots which made the most growth in the first three weeks were the first to become acid.

SUMMARY

1. After eight to twelve weeks' incubation the weights of pellicles of *B. tuberculosis*, human type, from glycerinated beef broth, which contained 20 mgm. of ferric sulphate per 100 cc., averaged 41 per cent more than the weights of pellicles from control broths which contained no added iron. However, during the first few weeks the bacteria grew more slowly upon iron than upon control media.

2. The growth of the bacteria was influenced favorably by ferric sulphate in media of pH values ranging from 5.9 to 8+. The weights of bacteria obtained from iron media of varying pH value were, within the range studied (5.9–8+), approximately the same, and were in all cases greater than the weights from control cultures of pH value 7.3.

3. The favorable influence of the iron was not dependent upon the kind of iron salt added.

4. The most favorable concentration of iron appeared to be from 0.01 to 0.03 gm. of ferric sulphate per 100 cc.

5. The effect of the iron was not influenced by the nature of the added salts, KH_2PO_4 and NaCl.

6. In general form the reaction curves of the control cultures resembled that regarded by Smith as characteristic of the human type of bacteria, and the reaction curve of the iron cultures resembled, at least for the greater part of the period of cultivation, that regarded by Smith as characteristic of the bovine type.

The writer wishes to acknowledge his indebtedness to Dr. M. Dorset, Chief of the Biochemic Division, upon whose suggestion and under whose supervision this work was done.

REFERENCES

(1) SAUTON, B.: Sur la nutrition du bacille tuberculeux, Compt. Rend. Acad. d. Sci., 1912, clv, 860.

(2) CALMETTE, A.: L'Infection bacillaire et la tuberculose, 2nd Ed., Paris, 1922, p. 39.

(3) Borrel, A., deCoulon, A., Boez, L., and Quimaud, J.: Milieux synthetiques pour la culture du bacille tuberculeux, Compt. Rend. Soc. d. Biol., 1922, lxxxvi, 388.
(4) Frouin, A., and Guillaumie, M.: Nutrition minerale du bacille tuberculeux, Ibid., 1923, lxxxix, 382.
(5) Frouin, A., and Guillaumie, M.: Action des sels de fer sur le rendement en poids du bacille tuberculeux, Ibid., 1924, xc, 831.
(6) Smith, T.: The reaction curve of the human and bovine type of the tubercle bacillus in glycerine bouillon, J. Med. Res., 1910, xxiii, 185.

AN INVESTIGATION OF THE ACID-FASTNESS OF TUBERCLE BACILLI[1,2]

B. SUYENAGA

This work was taken up at first as part of an immunological study to see whether it is possible to obtain an entirely non-acid-fast culture from a saprophytic strain of tubercle bacilli.

Tubercle bacilli have been thought by many workers to be non-acid-fast in their youngest phase. This opinion was first expressed by Ehrlich in 1884 in his study of the tinctorial character of tubercle bacilli. Marmorek reported a rapidly growing strain which in broth culture contained acid-fast bacilli in the central, and non-acid-fast bacilli in the peripheral part of the bacillary film. Further, by frequent transfer from the peripheral zone of growth, he could obtain a strain of non-acid-fast bacilli, called by him *primäre Bacillen*. Klein, in the same year, 1900, published a report of a twelve-year-old strain which contained many non-acid-fast elements. He also was able to demonstrate non-acid-fast bacilli in young cultures of other strains of virulent tubercle bacilli. Tint and Breskman, who made a laborious investigation on the variability of the acid-fastness of tubercle bacilli, using strains three months to sixteen years old after isolation, asserted that young cultures of all strains showed reduced acid-fastness. In this work they concluded that the older strains are the more acid-fast. However, it has been generally accepted that the growth of the tubercle bacillus becomes accelerated, its acid-fastness reduced, and its virulence attenuated after many years on artificial media. There is a strain of Saranac Laboratory tubercle bacilli called *R1*, isolated in 1891, which still grows slowly although only slightly virulent. A culture attributed to Farran is said to have been isolated from an inoculated guinea pig as a non-acid-fast strain in the beginning.

[1] From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago.

[2] A summary of this study was read before the annual meeting of the National Tuberculosis Association, 1919, and was also published in the AMERICAN REVIEW OF TUBERCULOSIS, 1919, iii, 473.

Wherry, working with a saprophytic strain of acid-fast bacilli, succeeded in robbing them of their acid-fastness by using media of poor nutritive value. This strain of acid-fast bacilli, which I used also in this work, was originally isolated in Robert Koch's laboratory and was brought to this country by Dr. V. C. Vaughan in 1888 and is termed *K1*. It is now nonvirulent, contains many non-acid-fast as well as acid-fast elements, and grows luxuriantly and rapidly on plain agar, although it was the same as the ordinary strains of tubercle bacilli in every respect at first. Wherry used, besides various media of definite chemical composition, an amoeba medium, and slants of 2 per cent agar dissolved in distilled water, which might be called non-nutrient agar. The amoeba medium consists of 2 per cent agar, 0.03 per cent salt and 0.03 per cent beef extract, without peptone, and is 1 per cent alkaline to phenolphthalein. The culture of K1 on these two kinds of medium became non-acid-fast after the fifth week, being transferred about every two days.

From the literature as reviewed above, a non-acid-fast or much less acid-fast strain might be expected from the K1 culture if we transfer its younger growth rapidly to new media through many generations. Growing so rapidly, the period of non-acid-fastness of K1 may be shorter than in the virulent strains. But the opposite is probably true, for its acid-fastness was found to be much reduced. In our attempt to obtain a non-acid-fast strain, the frequent transfer of the young culture of K1 through many generations was adopted as one of several methods used in this work. Another method for the same purpose was to alter the reaction of the media on which K1 is grown. Sörensen, using various other bacilli, showed that the reaction of the media influenced the activity of the lipase which is present in tubercle bacilli. According to him, a certain height of acidity stopped the action of lipase while it was favored by alkalinity. Besides the above two methods, the methods of Wherry, who succeeded in getting a non-acid-fast culture out of K1, were repeated.

The main part of this work was performed by keeping the culture of K1 on various media and staining the smears from them at various periods, controlling with the ordinary culture of this strain on our standard media, and one or two virulent strains of tubercle bacilli. The best to be done in such work as this is to accumulate as many tests as possible under certain rules and uniform conditions to see whether some conclusive result will eventuate.

EXPERIMENTAL DATA

Our standard culture medium for keeping the tubercle bacillus and other acid-fast bacilli is 5 per cent glycerol agar of 0.8 per cent acidity to phenolphthalein. Rapid transplantation was made in the effort to get a non-acid-fast culture of K1 on this medium. Media of various reactions were used, having otherwise the same composition as the laboratory glycerol agar, about 1.5 per cent acidity, and 1 and 2 per cent alkalinity. The non-nutrient media and amoeba medium used by Wherry were prepared as previously stated. Musgrave and Clegg mention, in regard to making of the amoeba medium, that it is usually necessary to start with an initial alkalinity of 1.5 per cent to obtain a final reaction of 1 per cent alkalinity to phenolphthalein. The agar medium of high acidity was more difficult to prepare. About 1.5 per cent acid reaction was the maximum obtained before the agar began to be dissolved.

At first cultures of K1 on all these media were transferred daily in preliminary experiments, but later, when the work was carried on more systematically, transfers were made about every two days. The transplantation was always made from the edge of colonies, which can be regarded as the younger part of the growth and which appears more watery than the middle whitish part. Transfers were continued on through many generations, and tested as to acid-fastness at various stages. Two series each for amoeba media and non-nutrient agar were also run. These media were inoculated in both series with the rapid transfer culture at the generations then reached.

Microscopic specimens were made from the edge of two-day-old cultures, but the material was taken from whole colonies from amoeba media and non-nutrient agar, owing to the scarcity of growth on these two kinds of media.

Smears of the cultures were made with sterilized distilled water on well-cleaned cover-slips as uniformly as possible. Later, a stock bacillary suspension was made of each colony, at first on a slide, and from this several cover-slip specimens were made. These were air-dried, and fixed by passing through a gas flame three times. The cover-slip smears from various cultures made for the same duration of exposure to acid decolorization constituted a set, and these were placed in a Petri dish which has four compartments divided by a cross of glass rods. This was filled with carbolfuchsin and heated on asbestos by a

small gas flame. When the carbolfuchsin steamed, the specimens were placed in it and kept for three minutes. Then after being washed thoroughly with distilled water, they were put into 14 per cent nitric acid solution (20 cc. of concentrated nitric acid of 70 per cent acidity and 80 cc. of water) for the intended duration and washed with water. Then they, all alike, were put into 70 per cent alcohol for three minutes. For the sake of convenience, these specimens were handled one after another at intervals of 30 seconds successively. After washing they were counterstained with Löffler's methylene blue, diluted 1:3. For washing, distilled water was used.

The durations of exposure to acid were 10 and 30 seconds, and 1, 2, 3, 5, 8, 10, 15, 30 and 60 minutes; but all of these durations were not employed in every test. Each test usually took up three, four or five durations from the above list for examining the smears from various cultures. And many tests with suitable combinations were accumulated to secure a fair review of the results of all tests.

On thorough examination of the specimens it was often found that bacilli of the same tinctorial character segregated themselves. It would therefore be a great mistake to judge a specimen by partial examination.

Besides the Ziehl-Neelsen method, the Gram stain and the simple methylene-blue stain were also tried on these cultures, and virulent strains of tubercle bacilli were used as controls. The methylene-blue stain used for this was as follows: 10 cc. alcohol-saturated methylene blue plus 40 cc. distilled water.

The K1 culture contains many non-acid-fast bacilli. Therefore, unless some remarkable change develops, we cannot be sure of an altered acid-fast nature of this culture. To clear the results of this work, five degrees of acid-fastness were standardized by the following classification:

I. Still many individual bacilli holding the fuchsin stain.
II. Overwhelming majority of individuals decolorized or blue-stained, although the bacillary masses hold the stain.
III. All individuals and majority of masses decolorized or blue-stained.
IV. All decolorized or blue-stained.
V. All well blue-stained.

All results of the tests were tabulated according to the above classification, and the following description is the result of analyzing these tables.

THE RESULTS

1. The stock culture of K1: This is kept by being transferred every two or three weeks in this laboratory, and it was rejuvenated by fresh transplantation before the staining tests were made on it. It takes about three days to get as good growth as a twenty-four-hour-old culture of other ordinary bacteria. The colony is slightly whitish and granular or wrinkled. When older, it becomes whiter and more granular and wrinkled.

Ten tests were made on the rejuvenated cultures when they were about two days old.

Exposure to the acid for 3 and 5 minutes seems to decolorize a little but this is not complete even after 15 minutes' exposure.

2. Virulent strains of tubercle bacilli. Strain A: Seven tests on cultures from 13 days to 33 days old were made. The smears for staining were taken from an opaque suspension which was filtered through a layer of cotton or paper. It holds the stain perfectly after exposure to the acid up to 5 minutes, and it begins to be affected after 8 minutes. From 15 minutes to one hour the stain is increasingly influenced, but sometimes it is held well even after one hour. The bacilli take the blue stain very little when counterstained after being decolorized with acid, and the color is brownish.

Strain B: Two tests on 14 and 26-day-old cultures were made. The results were about the same as in the case of strain A.

3. The rapid-transfer culture: In about 19 months 334 transfers were made. At about the 150th generation this culture already showed more general membranous development, and was less colored and more watery than the stock culture of the same age. At about the 280th generation, besides these differences from the stock culture, the colonies were more wet, but were not much different in their speed of growth. At about the 330th generation the color is not different but the growth is still more membranous and wrinkled, and it seems now to have a little better growth. These differences between the stock culture and the rapid-transfer culture become less or disappear when the cultures from both sources become older.

Twenty-two staining tests after the 216th generation were made. Five minutes' exposure to the acid decolorizes the stain to a considerable extent, but there are some cases in which one hour failed to decolorize it much. However, especially after the 326th generation, 15 minutes'

treatment with acid decolorized almost entirely, and the decolorized bacilli stained blue fairly well. In some tests before the 150th generation no definite difference between this and the stock culture could be noted.

4. Culture on 1 per cent alkaline glycerol agar: A good granular growth was obtained. Nine staining tests were made between the 19th and 73rd generations. The form of the microörganism was bacillary, being more or less granular. Three and 5 minutes' exposure to the acid seemed to have some decolorizing influence, but not much even after 8 minutes in some cases.

5. Culture on 2 per cent alkaline glycerol agar: About the same growth was obtained as above. Eight tests were made between the 71st and 64th generations. The bacillary form was preserved. No remarkable reduction of acid-fastness was obtained even by 5 or 8 minutes' treatment with acid.

6. Culture on 1.5 per cent acid glycerol agar: Beautifully white colonies of rather slow development were obtained. Several tests were made on the 1st and 8th generations with cultures from 15 hours to 2 weeks old. The appearance and development of the colony suggested that this culture might be more acid-fast than the other cultures, but this was not the case. The reduced acid-fastness, also, was not found to be constant.

7. Culture on non-nutrient agar: Granular or diffuse colonies with poor, thin development were obtained. Microscopically, coccoid forms prevailed, often mixed with many granular bacillary forms.

This culture and the amoeba-medium culture showed a curve in the degree of alteration of acid-fast stain. Two series were run for each medium.

Series I: Six tests were made. After the 25th generation the transfer became irregular, but cultures 2 days old were always taken for staining tests. At the 77th generation 5 minutes decolorized the bacilli totally, so that they stained well with methylene blue, although, curiously, 7 minutes' acid treatment still left many holding the fuchsin stain. From the 17th to the 34th generation, 3 or 5 minutes' acid treatment decolorized them, so that in most cases they stained well with methylene blue. But at the 48th and 49th generations they seemed to be more acid-fast, 3 minutes' acid treatment being unable to influence the stain remarkably. This also was the case with the culture on amoeba medium. Therefore K1 culture, after losing its acid-fastness gradually

on these nutritionally poor media, became adapted so that it could synthesize the acid-fast substance on them.

Series II: After transfer every 2 days until the 36th generation, the culture was transferred every 4 days for about a month. During this period no staining tests were made. Then transfer every 2 days was resumed and the staining tests begun again. The 7th and 8th generations showed remarkable reduction in acid-fastness when treated with acid for 5 and 8 minutes, but 3 minutes' treatment failed to decolorize. In the 8th generation 15 minutes' treatment failed to decolorize. Although the cultures around the 20th generation are about the same as the above, perfect decolorization and good blue stain resulted at the 24th and 25th generations, in most specimens even by 10 seconds of acid treatment. In 4 tests on decolorization from the 54th to the 59th generations, the culture was found to be more acid-fast, although 15 minutes' acid treatment produced fairly thorough decolorization.

8. Culture on amoeba media: This gave a thin and diffuse, but better growth than the cultures on the non-nutrient media. Microscopically, mostly granular bacillary forms were found. The granules in these bacilli often hold the stain well and they often appear as coccoids.

Series I: Six tests were made. In the 11th generation already a remarkable reduction of acid-fastness was found. From the 17th to the 38th generations the reduction was so marked that, in one case, 30 seconds' acid treatment decolorized the bacilli perfectly. At the 58th and 59th generations the bacilli seemed to be more acid-fast.

Series II: The transfers were continued as in the second series on non-nutrient agar. Ten tests were made. With apparently gradual decrease of acid-fastness this culture produced quite non-acid-fast and well blue-stained bacilli by 3, 8, and 15 minutes' decolorization at the 25th generation. At the 54th and 55th generations they were also decolorized, and blue-stained well after 5 and 15 minutes' acid treatment, but 30 seconds left many bacilli holding the stain. The 58th and 59th generations were somewhat more acid-fast.

9. Other bacteria: Diphtheria bacilli, Streptococcus pyogenes and colon bacilli were employed to see whether there is any acid-fastness in ordinary bacteria. The colonies used were all 36 to 40 hours old. The time for the exposure to acid was 10, 30 seconds, 1 and 2 minutes. Then all were treated by 70 per cent alcohol for 3 minutes.

All of them were decolorized perfectly, even by 10 seconds' acid

treatment, and took the blue stain. Only diphtheria bacilli retained a slight pink tone in some very thick masses in the 2 minutes' test.

GRAM STAIN OF THE ACID-FAST BACILLI

K1 culture and cultures of virulent tubercle bacilli contain many or a majority of gram-positive bacilli. It is to be noted that the substance responsible for the acid-fastness is regarded also as gram-positive (Tamura). But there must be some other substance in the acid-fast bacilli which is also gram-positive and not acid-fast. For when we succeeded in robbing K1 cultures of acid-fastness, we found that they were still gram-positive. The more intense Gram stain of tubercle bacilli than their acid-fast stain is demonstrable also with crushed tubercle bacilli. The acid-fast stain is almost covered up by its counterstain in crushed tubercle bacilli, while the Gram stain is taken very strongly, suggesting that the crushing procedure merely liberates more gram-positive substance from the bacillary bodies.[3] It is true, however, that the coccoid forms of K1 on the media of poor nutrition appear both acid fast and gram-positive. They were often found as granules, retaining their acid-fast nature, in the much less acid-fast or non-acid-fast bacillary bodies.

SIMPLE METHYLENE-BLUE STAIN

Rabinowitsch showed that tubercle bacilli could be stained with strong watery solutions of methylene blue. DeWitt and Sherman also stained tubercle bacilli with aqueous (1 per cent) and alcoholic solutions of methylene blue, in 24 hours, and even in 30 minutes. In my trial the staining of virulent tubercle bacilli with the watery methylene blue, as prescribed above, was irregular. In some cases they took the stain fairly well. Probably it is true that a young culture of an old strain is best stained by methylene blue. K1 cultures on the various media used are well stained by watery solutions of methylene blue.

DISCUSSION

The generally accepted idea that tubercle bacilli become more or less saprophytic, and grow luxuriantly after many years on artificial media, seems to need more support. The strain of A. Marmorek,

[3] Suyenaga, B.: *Trans. Nat. Tuberc. Assoc., 1921.*

which was only slightly acid-fast, did not form the characteristic film on broth. The film of tubercle bacilli on broth has no such concentric structure as that of Marmorek. It is merely wavy and membranous. On the other hand, saprophytic acid-fast microörganisms are not rare which would cause contamination of laboratory stock cultures of tubercle bacilli. The author found a strain of them growing spontaneously on Petroff media and another was isolated from a guinea pig. There are several reports of such microörganisms by others. All of these grow at both room and body temperature.

In regard to the non-acid-fastness of young tubercle bacilli, this investigation, especially that part of the work with the rapid-transfer culture, indicates that this is probably true. The finding of Tint and Breskman that the young culture of a strain is less acid-fast is in agreement with this observation. However, their conclusion that the older strains are more acid-fast is based on an investigation in which they used, for their staining tests, four strains of tubercle bacilli, varied in the lengths of their incubation from two to four weeks. De Schweinitz and Dorset, in their chemical study of tubercle bacilli, showed that an old, attenuated human strain contained more fatty substance than younger virulent human strains and four other younger strains from animals and birds. In their work cultures of the same age in the incubator were not used but the cultures differed from two to four months. Therefore neither the greater acid-fastness of the older strain of tubercle bacilli nor the saprophytism of the old strain of tubercle bacilli seems to be established yet by sufficiently thorough observations.

CONCLUSIONS

1. By rapid transfer and rapid growth of the younger borders of colonies of a saprophytic strain of tubercle bacilli, I was unable to establish a non-acid-fast strain. However, after over 300 transfers the acid-fastness of the culture was considerably reduced.

2. The non-nutrient medium and the amoeba medium used by Wherry practically robbed the culture of K1 of its acid-fastness. After reaching the lowest stage of acid-fastness, bacilli on these media may again become more acid-fast.

3. The reactions of culture media, within the limits used in these experiments, seem not to have a marked influence on the acid-fastness of this saprophytic strain.

BIBLIOGRAPHY

EHRLICH: Charité-Ann. 1884, xi, 123.
MARMOREK: Ztschr. f. Tub. u. Heilst., 1900, i, 444.
KLEIN: Centralbl. f. Bak., 1900, xxviii, 111.
TINT AND BRESKMAN: J. A. M. A., 1910, liv, 1591.
DE SCHWEINITZ AND DORSET: Centralbl. f. Bak., 1902, xxxii, No. 3.
WHERRY: J. Inf. Dis., 1913, xiii, 144.
RABINOWITSCH: Ztschr. f. Hyg., 1897, xxvi, 90.
DEWITT: J. Inf. Dis., 1913, xii, 68.
SHERMAN: J. Inf. Dis., 1913, xii, 249.
MUSGRAVE AND CLEGG: Publ. of Govt. Lab. Manila, No. 18, 1904.
TAMURA: Ztschr. f. Phys. Chem., 1913, lxxxvii, No. 2.

A METHOD FOR TRANSFERRING TUBERCLE BACILLI FROM SOLID TO LIQUID CULTURE MEDIA[1]

ALBERT LUTZ

To transfer tubercle bacilli from solid media to solid media is quite simple and is easily accomplished. To transfer from liquid to liquid media is also readily done. Transfer is easily made from liquid to solid media, but it is often a difficult task to seed a broth culture with bacilli from a tube culture, as the dry warty and lumpy growth is prone to sink to the bottom of the fluid.

Various methods have been tried with more or less success. Small square films of thin paper floated upon the surface of the broth have been used to support the new transplant until it should grow out over the edge and on to the broth. Cubes of solid medium with a thickness corresponding to the depth of the liquid have been placed in media bottles and seeded with bacilli, or bits of cork have been used to support squares of agar, seeded with bacilli, on the broth. Then to the bottles has been added the requisite amount of broth and the bottle incubated. The bacilli will grow from the solid-medium cube and on to the surface of the broth. The addition of a small amount of broth to solid-medium tubes, on which were planted tubercle bacilli has been used, and successfully. The growth spreads from the solid medium, as a pellicle on top of the broth. This may be transferred to a bottle of broth.

A most satisfactory method is the one about to be described. Solid-medium slants are made in 100-cc. Erlenmeyer flasks by pouring in 50 cc. of glycerine agar while in a liquid state. This is properly sterilized, and the flask is placed in such a position as to allow the medium to harden into a slant that runs from just below the mouth to the bottom of the flask. When hardening has taken place, 15 cc. of sterilized glycerine broth are added, and the flask is sealed and kept in an upright position and stored in a dark closet until needed.

When transplants are to be made the slant is heavily planted with

[1] From the Kenneth Dows Tuberculosis Research Fund of the Johns Hopkins Hospital and University.

bacilli down to the fluid level, and is then incubated. Better growth occurs if the paraffined plug is not sealed into the flask, but is placed

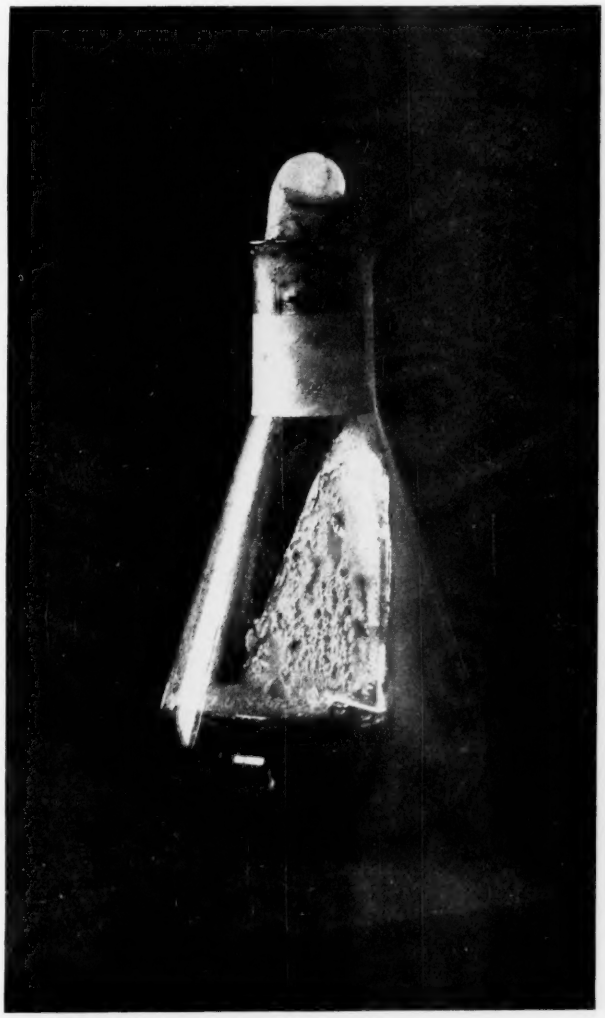


FIG. 1. RETOUCHED PHOTOGRAPH OF COMBINATION SOLID AND LIQUID CULTURE OF TUBERCLE BACILLI

Heavy growth of bacilli covers the surface of the solid medium and extends apron-like on the broth surface as an extensive layer. Bacilli taken from this broth surface are easily floated on to the surface of liquid cultures.

in lightly for two days. The growing bacilli cover the solid surface, and by continuity spread out over the surface of the broth, so that

usually by the end of three weeks the entire liquid and most of the solid surface are covered with a continuous sheet of bacillary growth. At times the growth will spread to the broth but poorly, but in these circumstances a pellicle of bacilli may be pushed off from the solid surface and allowed to fall on to the liquid surface. This usually grows very promptly and satisfactorily. Once there is growth upon the broth, transfer to other broth cultures becomes easy.

A fullgrown four-weeks-old culture is shown in the accompanying figure 1.

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TUBERCULOSIS IN FILIPINOS[1,2]

G. R. CALLENDER AND M. W. HALL[3]

A high degree of tuberculization and a high incidence of manifest tuberculous disease among the natives of the Philippines has been generally conceded. A few general statements to this effect have appeared in the literature, but no definite studies of the subject have been made other than such as are parts of the reports of the Philippine Health Service and the Census Report of 1918. These official reports are based on Health Service data and have not hitherto been carefully reviewed with reference to the significance of the age distribution of the death rates nor the ratios of the pathologico-anatomical types. A few publications have dealt with special phases of the subject. After the Out-Patient Clinic of the Philippine General Hospital had been in existence sufficient time to accumulate a satisfactory series of cases for study, Musgrave and Sison (1910) reported their observations on 1057 patients presenting themselves at the tuberculosis clinic, 914 of whom they considered to have tuberculous lesions in the lungs. This number represented 22 per cent of all patients applying for treatment to the entire Out-Patient Clinic. We have found two other reports which give definite data: that of Gilman (1908) on the incidence of tuberculous lesions in a small series of autopsies in which no special search was made for the small lymph-node localizations, and a study of the lesions found in 500 autopsies on children by Mendoza-Guazon (1917). These autopsy studies give data on the distribution of the lesions, but no accurate idea of the total incidence, inasmuch as no special search was made. Of the general statements, Chamberlain, Whitmore and others have given their opinion that the disease is prevalent, and their statements are based on their personal observations, backed to some extent, at least, by their knowledge of the health reports. Bushnell (1920) studied the statistics and literature, and came to the conclusion that the Filipinos as a whole

[1] From the United States Army Medical Department Research Board.

[2] This paper is based on (1) a tuberculosis survey of Filipino and American troops in the Philippine Islands; (2) a study of the vital statistics of the Philippine Health Service; (3) a review of the records of the Philippine General Hospital at Manila and its branch at Cebu; and (4) an analysis of the tuberculous lesions in 10,000 autopsies from the Department of Pathology, College of Medicine, University of the Philippines.

[3] Major M. W. Hall assisted in the arrangement of the statistical data and revised the discussion of them.

were well tuberculized and that the available data indicated an incidence of the disease which would be expected in such a well-tuberculized community with poor hygiene and sanitation. The factor of poor hygiene and sanitation was first stressed outside of official Health Service reports by Musgrave and Sison (1910) in their paper, and covers the situation very well. They write:

It may be stated in general that conditions favorable for the spread of tuberculosis are practically perfect in this Archipelago. Overcrowding in unsanitary houses, located too close together in unsanitary places and closed at night, is all but universally prevalent. Our records show many instances of from six to ten or more people living and sleeping in a single room, with from one to several individuals among them suffering from advanced pulmonary tuberculosis. Children are born and reared under these circumstances and very frequently nursed by their tubercular mothers. . . . . The disease is very prevalent among all classes of people whose occupations are such as to keep them in close relationship with healthy persons. In this class may be mentioned street-car conductors and motormen, carriage drivers, *house servants, cooks and nurses of infants.*[4] During the last year we have caused the dismissal of no less than fifteen women suffering from phthisis who were caring for and, in some instances, sleeping in the same room with the children of their employers. The particular dangers from promiscuous coughing and expectoration are unknown to the majority of the patients, and these acts are practised unrestrictedly at all times and in all places. Poor food and lack of exercise are very potent predisposing factors to tuberculosis among the poor of these islands.

Of particular interest are Musgrave and Sison's tables on the family history which are reproduced below:

TABLE II (Musgrave and Sison)

*Showing family history**

FAMILY	TOTAL NUMBER	NUMBER LIVING	NUMBER DEAD	NUMBER TUBERCULAR	PER CENT TUBERCULAR
Father	914	256	258	169	18
Mother	914	327	537	272	29
Paternal uncles	466	165	301	155	33
Paternal aunts	318	98	220	104	32
Maternal uncles	401	126	295	135	33
Maternal aunts	465	155	310	161	34
Brothers	2,094	937	1,157	167	7
Sisters	1,866	914	952	135	7
Consort	601	443	158	104	17
Children	1,920	887	1,023	83	4

Family history positive in 88 per cent of all cases.

* Numerical inconsistencies are those of original authors.

[4] Italics Callender and Hall's.

TABLE III (Musgrave and Sison)
Showing tuberculosis in ascendants

	WILLIAMS	SOLLY	OSLER	MANILA
Total cases	1000	250	427	914
	per cent	*per cent*	*per cent*	*per cent*
Parental	12	28	24	47
Collateral	34	19		35

Except for the health reports, which are based on the population as a whole and on autopsy reports up to the year 1924, and which are studied in some detail in the following pages, the papers quoted represent the sum of published information on the subject of tuberculosis in the Filipino. In the course of the study here presented the following factors have been investigated with the attempt to correlate the various findings in such a way as to give a comprehensive idea of actual conditions:

1. A study of the mortality statistics contained in the reports and records of the Philippine Health Service, using the data of the U. S. Census Bureau for comparison.
2. A study of the incidence rates in the Philippine General Hospital.
3. A study of the tuberculosis incidence in a series of autopsies 10,000 in number.
4. A physical survey of a group of Filipinos and comparison of the results with those from a group of Americans.

It was not practicable to utilize the tuberculin reaction, and it was not considered so important as the methods used in a study of the general population.

MORTALITY STATISTICS

The basis of this section of the report is a study of the statistics of the Health Department from the standpoint of the age incidence of the disease and the age distribution of the pathologico-anatomical types of the disease. This distribution, as is shown in the works of Bushnell, Calmette, and German writers, has an epidemiological significance, and can be used as a basis for conclusions as to the prevalence of the disease and the degree of exposure and tuberculization of the population.

To explain this, it should be said that there are three types of the disease, distinguished primarily by the varying resistance of persons attacked and secondarily by the size of the infecting dose or the rapidity

with which the threshold of infection is passed. These types are first, that arising in the nonimmunized subject from a massive primary infection, and this is called *primary tuberculosis*. In the tuberculized population, that is, one well-exposed to infection, this type is seen only in children who receive an overdose before they have developed an immunity of any degree. It is characterized by an acute type of disease, either miliary or massive and more or less disseminated infection, and not infrequently confined for the greater part to the lungs. There is also sometimes a disease of fulminant type which is a general tuberculous septicemia, and with few or no recognizable localizations or tubercle formation. To this type Landouzy gave the name *Typho-bacillose* (Calmette, 1923). Lymph nodes are undoubtedly the distributing centre for this type, bacilli entering by way of the respiratory or digestive tracts. There is, except in the most fulminant *typho-bacillose* types, a primary adenopathy which is followed, if the patient lives long enough, by secondary lymph-node enlargements. This primary type is also seen in the adult who has never before been in contact with the virus and is exposed to large doses before he can develop any immunity. This type of the disease has wrought fearful havoc in tropical islands, particularly those chosen as health resorts for the tuberculous because of the absence of the disease when first visited by explorers (Bushnell, 1920).

The second type is that found in persons who have developed a low-grade or imperfect immunity, and is characterized by lymph-node tuberculosis of considerable extent and involvement of the peritoneum, viscera and bones, and, when fatal, by termination from meningeal tuberculosis. The most familiar type is the cervical lymph-node involvement, the "scrofula" of youth.

The third type is the tuberculosis of more or less chronic type, developing in the rather well-immunized individual through some decrease in resistance allowing a focus hitherto under control to extend. Just why this occurs we have ideas based on general observations, but the type is distinct, although the younger the person affected the more rapid, as a general rule, is the course of the disease.

This type is the ordinary phthisis of slow progress, and is characterized by marked resistance to the extension of the disease by means of fibrosis. Lymph nodes, while larger than normal, do not show the enormous enlargements of the primary and secondary types, though occasionally those draining an active focus are large and caseous.

The proportion of the third type indicates, in a general way, the degree

of tuberculization or resistance to tuberculosis of the population. The proportion of cases of type 1 in other than children indicates the degree of nontuberculization, while any great number of the second type indicates overdosage in those who have not developed much immunity. A high tuberculosis mortality in children, or a large number of cases of the second type of the disease in a community with a preponderance of the third type, indicates poor hygiene and lack of use of preventive measures, and is an index of the health standard of the communities in which it occurs.

Bone and joint tuberculosis, in general, increases as preventive measures decrease, but there are other factors little understood which do not allow these lesions to be considered as important from the epidemiological standpoint as those described above.

In the study of any group of statistical data one is first concerned with its relative reliability. It is to be admitted that no large mass of statistics is entirely accurate. The means of collection of statistics, even in the most highly developed communities, leave much to be desired, and this is especially true of vital statistics in which decisions as to the actual cause of death must necessarily be made by physicians of widely varying diagnostic ability. In the 1920 *Mortality Statistics* published by the U. S. Bureau of the Census we find recorded certain statements as to the reliability of the data contained therein. When reports did not appear to be correct, or the principal cause of death was not defined, queries were sent out to the reporting physicians. Of 23,925 such cases, the diagnosis was changed in 48.1 per cent. The percentage of reported causes of death thus queried in the U. S. Registration Area for 1920 was 3.7, and varied in different states from 0.5 per cent to 16.9 per cent. Inasmuch as the reports not queried probably also contained a considerable proportion of errors, though of a nature not readily recognizable by those engaged in their tabulation, it is readily seen that the reports of the Bureau of the Census are not entirely accurate. Such accuracy is impossible under present conditions anywhere, and particularly in a community such as the Philippine Islands, whose development has been retarded for centuries.

In the Philippines, death reports are necessarily made in the majority of cases by nonmedical officials. The reports of the Philippine Health Service state that only about three-tenths (30 per cent) of the death certificates are made out by doctors of medicine. In Manila about half are made out by doctors, while, according to the census report of 1918,

from 80 per cent in some provinces to less than 10 per cent in others are made out by civil officials who write what from their lay knowledge seems most reasonable or take the diagnosis of relatives. In Antique Province, with about 150,000 inhabitants, there are two physicians, one a Health Service Officer on part time, and a second who devotes most of his time to other more gainful occupations. Of course, under such conditions the autopsy rate is very low. When it is remembered that many forms of tuberculosis, particularly the miliary, abdominal and meningeal types, are easily confused clinically with other and more familiar diseases, it is to be expected that many such cases are reported erroneously. Acute pulmonary tuberculosis in childhood is easily confused with broncho-pneumonia. These mistakes are made to some extent all over the world and are avoidable only by universal autopsy.

However, the conditions existent in the Islands undoubtedly lead to a degree of unreliability in the reporting of at least the less familiar forms of tuberculosis, which probably accounts for the slight use made of official statistics by earlier students of this subject. It is believed, however, that the figures themselves present certain internal evidence of reliability in the case of the more usual types of the disease, and that the errors present will usually be of omission rather than of overstatement of the number of cases of the disease. Moreover, among the cases reported the age distribution of mortality and of pathological types is not so subject to the sources of error mentioned, and it has accordingly seemed well worth while to study the age distribution of the mortality shown by the death certificates, and to approximate at least the percentage distribution of the pathologico-anatomical types at the different ages, in order to make the epidemiological deductions referred to above. From time to time, in the course of the discussion of the figures thus obtained, it seems necessary to explain apparent discrepancies or improbable findings by the assumption of error in the source of the statistics. When this occurs it should not be understood as a reflection on the diligence or enthusiasm of the officers of the Philippine Health Service, or of its director, Dr. Vicente de Jesus, through whose courtesy was made possible the preparation of certain data used herein.

With regard to the other factor necessary to the calculation of death rates, namely population figures, there appears some reason to believe that here, too, the situation is not entirely satisfactory. Censuses were taken under American authority in 1903 and in 1918. The total population recorded in the former census was 7,635,426. The 1918 census

showed 10,314,310. This is at the rate of over 2 per cent annual increase. The possible part played by immigration in this increase is negligible, as is shown by the figures for foreign-born inhabitants. During the period the rate for infant mortality was so high as to call for special comment by the health authorities (Census Office, 1921, pp. 1019 and 1106). That in spite of these unfavorable factors, the increase should have been at so high a rate appears improbable. Earlier enumerations of population showed a slower rate of increase. The increase of the Christian population between 1877 and 1887 was at the rate of 0.75 per cent per year; and between 1887 and 1903, 1.05 per cent per year (Census Office, 1921, p. 24). These considerations lead to the suspicion of a considerable overestimate of the population of the Islands, a condition which, if a fact, would result in published rates being lower than should be the case.

To return to the matter of reporting of deaths, it appears evident that certain large districts of the Archipelago are incompletely covered in this respect. The Division of Mindanao and Sulu, comprising the so called *Moro Provinces*, contains approximately one-tenth of the population of the Islands. The Census Report of 1918 gives 1087 deaths from tuberculosis in these provinces. The report of the Philippine Health Service for the same year gives for the same provinces 783 deaths from tuberculosis. These figures are 3.4 per cent and 3.1 per cent, respectively, of the total deaths reported for the Islands as a whole, 31,890 as given in the Census Report and 25,236 as reported by the Health Service. The census report shows only 5.5 of deaths from all causes as occurring in these provinces. As these provinces contain 10 per cent of the population of the Islands, we must assume from these figures either that the Moro Provinces have a far lower death rate than the Christian Provinces, or that reporting of deaths in the former is far from complete. The latter assumption seems much the more probable. That this is also the case in reports from districts inhabited by the pagan tribes of the mountains goes without saying. Elsewhere in the Islands deaths are probably very generally reported.

Two errors of considerable magnitude thus render most published death rates for the Islands as a whole open to doubt as to their correctness. One is the probability of an overestimate of population, and the other the practical certainty of a considerable understatement of the number of deaths in considerable portions of the country. Both causes, if operative, would tend to decrease rather than to increase the rate ob-

tained, and hence the figures for Philippine death rates should be regarded as too small rather than too large, and the prevalence of tuberculosis as greater rather than less than the figures would indicate.

In the City of Manila deaths are probably practically all reported and the population much more accurately known than is the case in the provinces. Hence rates for the City are much more satisfactory than those from other parts of the Islands. In the City of Manila, however, 10 per cent of the population and about 6 per cent of those dying of tuberculosis are Chinese and a certain small proportion are of other nationalities. These foreigners naturally show an age distribution different from that of the native population. Moreover, Manila is the principal medical centre of the Archipelago, as well as the centre of its political and educational activities. The former circumstance results in attracting to the city a large number of seriously ill persons, many of whom die here; the latter brings to the city a large transient population, usually of young adult age. Both these factors would prove disturbing in drawing epidemiological deductions from the total deaths in the city and from their distribution in age groups. For these reasons the figures (available only for recent years in the reports of the Health Service) for Filipino residents of Manila give the truest picture of conditions actually existing and will be those mainly depended upon in subsequent discussions. While the rates shown for the Islands, as a whole, are doubtless much lower than they should be, the age distribution of the deaths thus reported is so nearly the same as that seen in the Manila figures that deductions as to the degree of tuberculization, etc., drawn from the relatively exact Manila figures may fairly be extended to include the Filipinos as a whole. Bearing these considerations in mind, the following figures are presented, not for the purpose of exact comparison, but to give an idea of the magnitude of the tuberculosis problem in the Islands.

We first present the total death rates from tuberculosis for the Philippine Islands and for the City of Manila for the years 1911–1922 inclusive; and, for comparison, the rates for the U. S. Registration Area, cities, rural and total, and also the rates for the State of Virginia, white and colored, and for the City of Richmond. The rates for Virginia were chosen because the populations, white and colored, are both large enough to be representative and the rates for whites appraoch fairly closely those of the entire registration area.

These figures are presented in table 1, and are illustrated graphically

in charts 1 and 2. Chart 3 is copied from the *U. S. Public Health Service Reports*, and shows the trend of the mortality curve from tuber-

TABLE 1

*Comparison of death rates per 100,000 for tuberculosis (all) by years**

	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922
Philippine Islands*	241	234	214	223	224	230	248	336	285	250	250	249
Manila	571	558	496	486	527	562	586	800	636	628	578	561
Manila—Filipino residents only											526	509
Provinces, Philippine Islands								272	271	240	240	238
Registration Area Total	159	150	149	147	146	142	147	150	126	114		
Registration Area Rural	136	127	126	126	126	125	130	135	117	108		
Registration Area Cities	175	165	163	161	158	156	161	164	134	118		
Virginia, white			117	122	117	118	109	126	97	95		
Richmond, white	158	173	124	127	130	134	122	146	121	114		
Virginia, colored			276	308	328	302	308	316	272	256		
Richmond, colored	406	368	344	378	356	382	312	318	278	249		

* The figures for the Philippine Islands and for Manila were obtained from Vol. II, *Census of the Philippine Islands, 1918*, and from reports of the Philippine Health Service. Those of the United States were computed from the reports of the Census of 1920 and the Census Bureau's volume on *Vital Statistics* for the same year.

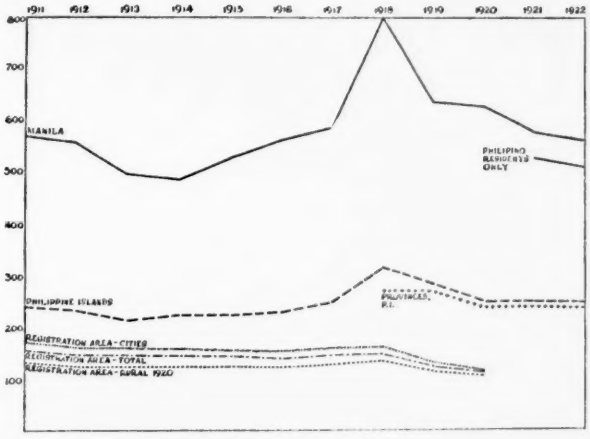

CHART 1. COMPARISON OF DEATH RATES PER 100,000 FOR TUBERCULOSIS (ALL) BY YEARS, SHOWING MANILA, PHILIPPINE ISLANDS AND UNITED STATES TOTAL REGISTRATION AREA

culosis in recent years in the United States and in Great Britain (Britten and Sydenstricker, 1922).

The figures of table 1 give an idea of the general prevalence of tuber-

culosis in the Islands as compared to the United States. The rates for the Philippines are consistently higher than those of the Registration Area, urban or rural, and since 1918 have been higher even than those

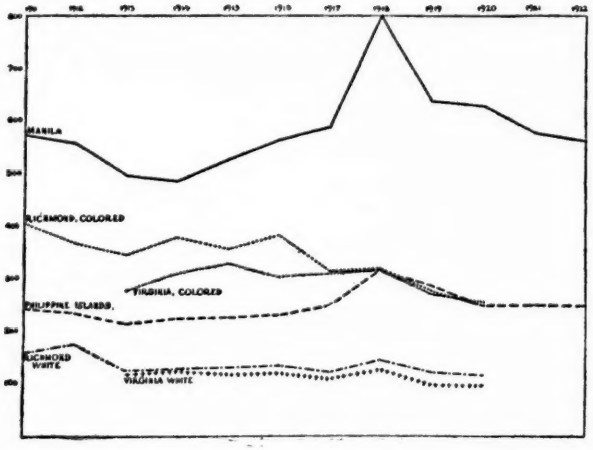

CHART 2. COMPARISON OF DEATH RATES PER 100,000 FOR TUBERCULOSIS (ALL) BY YEARS SHOWING MANILA, THE PHILIPPINE ISLANDS, VIRGINIA (WHITE AND COLORED) AND RICHMOND (WHITE AND COLORED)

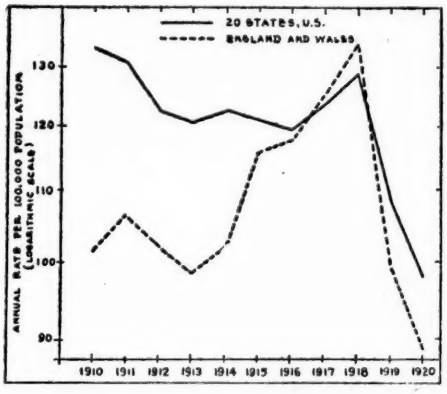

CHART 3. COMPARISON OF DEATH RATES PER 100,000 FOR TUBERCULOSIS (ALL) BY YEARS FOR TWENTY STATES, UNITED STATES, AND ENGLAND AND WALES (AFTER BRITTEN AND SYDENSTRICKER, 1922)

for the colored population of Virginia. The probability that rates for the Archipelago, as shown, are an understatement of the facts has already been indicated. This is borne out by the great disparity between these rates and the much more accurate figures for the City of Manila. No

such difference is seen between urban and rural rates in the United States, even in the susceptible colored race.

It is to be noted that the rates for all the American groups, like those published for the European countries, show a marked tendency to fall after the sharp rise accompanying the influenza epidemic of 1918. The reverse is true of the Philippine rates which, both for the Islands as a whole and for the City of Manila, show a higher average after the epidemic year than obtained previously. This rise is so strikingly out of keeping with the experience of the rest of the civilized world that it calls for special comment. There is found in death rates everywhere a sharp rise accompanying and, in some cases, slightly preceding the 1918 epidemic. Following this rise there has appeared elsewhere a very marked decrease of reported tuberculosis mortality to far below the former level (chart 3).

The explanation of this rise and fall is the subject of an article by Britten and Sydenstricker (1922), in which they incline to the belief that there is a rise independent of the influence of the influenza waves and possibly due to the economic conditions consequent to the war. It seems certain to us that the greater part of this rise is due to those waves of influenza which preceded the main epidemic, starting about 1914 and possibly aggravated by war conditions, as the increase began earlier in Europe than in America. This increase in reported deaths seems due to two conditions, first, the death of many phthisis cases as the result of intercurrent influenza, and secondly, erroneous diagnosis, the more protracted influenza cases with empyemata and suppurative processes in the lung being confused with tuberculosis. The subsequent fall is explained by the effect of the former of these causes in prematurely removing a large number of cases in which decrease would otherwise have occurred in the later period.

The causes that operated to prevent this fall in the Philippine tuberculosis death rate are not clear. The rate appears to have been rising before the onset of the epidemic, and the failure to fall, in conformity with the almost universal experience elsewhere, suggests the existence of some definite cause therefor. It is possible that better diagnosis accounts, to some extent, for the rise in the number of reported deaths, but it does not seem probable that this could account for the great difference shown between the upward tendency of the Filipino rates and the sharp depression in those of other countries. The difference may be explained in part by the influence of the epidemic occurrence of smallpox in the

years 1919–1921, but the rate shows no significant fall in 1922 when the epidemic had spent its force. It seems clear then that another cause must account for the actually increasing tuberculosis death rate in the Philippines while the rates in other countries are falling. The examination of the specific rates for the different age groups and the distribution of the different pathologico-anatomical types of the disease may lead to some conclusion as to the probable causes leading to this finding. The main points to be brought out by study of table 1 are the proof of the great prevalence of tuberculosis in the Islands and the fact that this prevalence appears to be on the increase in contrast with the experience in the rest of the civilized world.

It was stated above that a high tuberculosis mortality in children, occurring in a highly tuberculized population, was evidence of conditions leading to an unduly large proportion of massive infections by an overwhelming dose of the virus, before any immunity could be developed. Such conditions are those found in crowded populations in which precautions against the spread of infection are ignored. The degree of tuberculization of a community or population is estimated by the relative prevalence of the more chronic types of the disease and, in particular, the predominance of the chronic fibroid type of the pulmonary lesion. Inasmuch as the majority of tuberculosis deaths in those past early middle age are due to these more chronic forms of tuberculosis, a study of the specific death rates by age groups serves to give evidence both as to the tuberculosis death rate in childhood and the degree of tuberculization of the population studied. These rates for the Philippines and for Manila are shown in table 2 and plotted in chart 4. The figures for the U. S. Registration Area are included for comparison.

Like all rates, these figures are dependent for their value, not only upon accuracy in reporting of deaths, but also upon the exactness of the population estimates. For this reason reliance will not be placed upon the rates for the Philippines as a whole, although they are included in the tabulation. Fortunately for our purposes, the figures for the City of Manila may be regarded as much more satisfactory. The population figures cannot be far in error and the reporting of deaths is considered practically complete. Only the diagnosis remains as a considerable source of error and, in the case of a well-known condition such as tuberculosis, this also is doubtless sufficiently exact to give us a working basis. As will be seen later in the consideration of the age distribution of the various anatomical types of the disease, the figures themselves

contain internal evidence of substantial accuracy. Reference to chart 4, plotted from table 2, shows at a glance that the death rates for all age groups after childhood are enormously higher in the Filipino than is the case in the United States. The Manila figures are consistently higher than those for the Archipelago, but show such a remarkable degree of parallelism to those of the latter as to suggest that the differences are due to the diffculty in obtaining full and complete mortality statistics in the present stage of development of the Islands. The greatest disparity between these two sets of rates is found in those of early childhood. Just these deaths would most often fail of record in the less-advanced districts. The two sets agree, however, in showing a high rate after adolescence, a rate increasing as years advance, and thus affording evidence of a high degree of tuberculization of the race. The high proportion of the chronic fibroid phthisis is also brought out below in the consideration of the pathologico-anatomical types of the disease.

We may regard it as established, then, that the evidence shows a high degree of tuberculization of the Filipino race. The rates for childhood for the City of Manila are also relatively and absolutely high. It remains to be shown that they are high out of proportion to the general tuberculosis mortality. For this purpose table 3 has been prepared to show the percentage of the total deaths from tuberculosis that occurred in each age group. Such a classification is independent of population statistics and, with populations having a similar age grouping, is a particularly satisfactory method of bringing out the age distribution of deaths. When the age distribution differs materially, however, it is necessary to adjust these percentages to a standard age grouping by means of the specific rates for age groups. For purposes of this comparison, then, as shown in chart 5, the Manila rates have been adjusted to the age distribution of the white population of Viginia, as have those of the colored residents of Virginia, also shown for comparison.

This chart shows graphically the age distribution of deaths in three different races of differing degrees of tuberculization. The colored race in American has little resistance to tuberculosis. As a race, it has been in contact with this disease for only a few hundred years. The curve of mortality by age groups rises rapidly after early childhood and reaches its maximum in the 20–29 year group, when it is far higher than either of the others. It rapidly falls in the older groups and after forty is definitely lower than the rates for whites or for Filipinos. This is the curve of a little-tuberculized population. Below five, the rate is but little higher

TABLE 2

Rates per 100,000 of tuberculosis deaths by age groups

	LESS THAN 1 YEAR	1 YEAR	2–4 YEARS	5–9 YEARS	10–19 YEARS	20–29 YEARS	30–39 YEARS	40–49 YEARS	50–59 YEARS	60–69 YEARS	70 + YEARS
Philippine Islands, 1918	92	56.4	47.6	33.2	78	334	527	768	1,013	1,420	1,495
Virginia, colored, 1920	166	134	66	47	182	527	363	277	242	274	227
Virginia, white, 1920	68.7	48.4	18.8	11.8	27.2	135.3	135.7	129.2	173.8	214.7	303.6
United States Registration Area, 1920	108	72	33	18	60	175	162	147	146	159	165
Manila, Filipino only, 1918		385		316	286	847	1,230	1,360	2,000	2,495	2,470
Manila, Filipino residents, 1921		304		114	169	475	820	965	1,294	1,828	1,739
Manila, Filipino residents, 1922		317		118	181	493	672	894	1,445	1,669	1,592

TABLE 3

Percentage of total deaths from tuberculosis occurring in each age group

	LESS THAN 1 YEAR	1 YEAR	2–4 YEARS	5–9 YEARS	10–19 YEARS	20–29 YEARS	30–39 YEARS	40–49 YEARS	50–59 YEARS	60–69 YEARS	70 + YEARS
Registration Area, 1920	2.0	1.47	2.0	1.72	9.95	26.6	21.2	14.7	9.99	6.65	3.88
Virginia, white, 1920	1.75	1.23	1.42	1.49	6.15	23.8	18.7	14.2	12.4	10.5	8.34
Virginia, colored, 1920	1.53	1.13	1.88	2.39	16.5	35.92	18.06	11.27	5.83	3.92	1.92
Manila, 1920	0.8	1.5	3.8	4.55	6.55	22.66	20.00	17.07	12.2	7.52	3.36
Philippine Islands, 1918	1.57	1.75	3.61	2.46	7.65	20.35	21.11	17.93	12.48	7.49	3.5
Richmond, white, 1920	4.41	1.47	0.73	1.47	3.7	21.3	20.7	19.2	14.8	7.5	5.1
Richmond, colored, 1920	2.22	2.96	0.74	4.44	16.2	37.0	19.2	9.6	4.9	1.5	
Manila, Filipinos only, 1918	0.84	1.6	3.99	4.74	6.85	23.47	20.50	15.49	11.45	7.60	3.47
Manila, Filipino residents only, 1921		6.21		2.32	5.71	13.5	18.3	17.14	14.73	14.42	7.73
Manila, Filipino residents, only, 1922		6.7		2.5	6.7	14.4	15.5	16.4	17.0	13.6	7.3

than that of the whites of Virginia, but the shape of the curve implies that the disease is more acute, and therefore of shorter duration in negroes

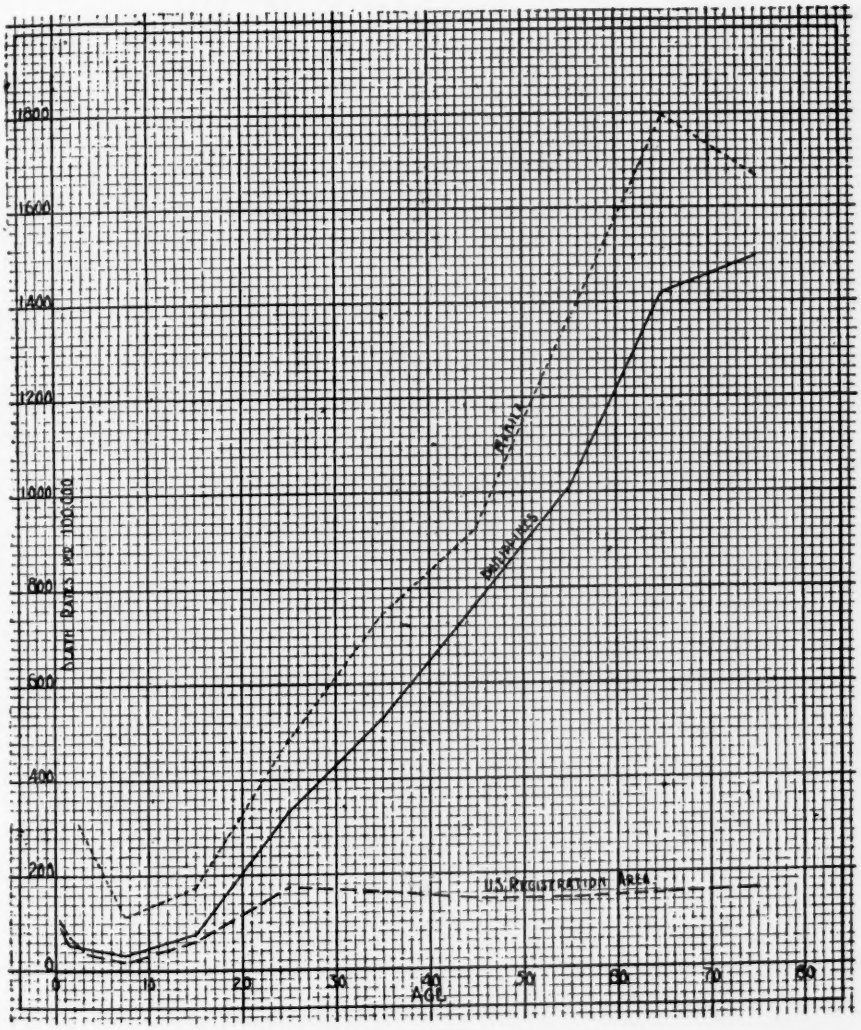

CHART 4. RATES PER 100,000 TUBERCULOSIS DEATHS BY AGE GROUPS FOR PHILIPPINE ISLANDS, 1918, MANILA (FILIPINO RESIDENTS) 1921–1922, AND UNITED STATES REGISTRATION AREA, 1920

than in the others. Those attacked are carried off more rapidly, and do not linger into the older age groups as chronic sufferers from consumption. The Filipino shows a totally different curve. The rise in mortality

after childhood is very slow, the maximum proportion of deaths occurring in the group 40–49. Even the extremely aged show a high proportion of tuberculous decedents. This is evidently the antithesis of the condition shown by the colored race. The chronic type of the disease predominates, and death is delayed to an advanced age.

The curve for the white race is definitely intermediate between the two described. It does not rise so soon nor so high after childhood as does the curve for the colored, but sooner and higher than that for the Filipino. It drops below the Filipino curve about age forty, but remains above the curve for the negro. Vertical lines have been erected in chart 5 at points

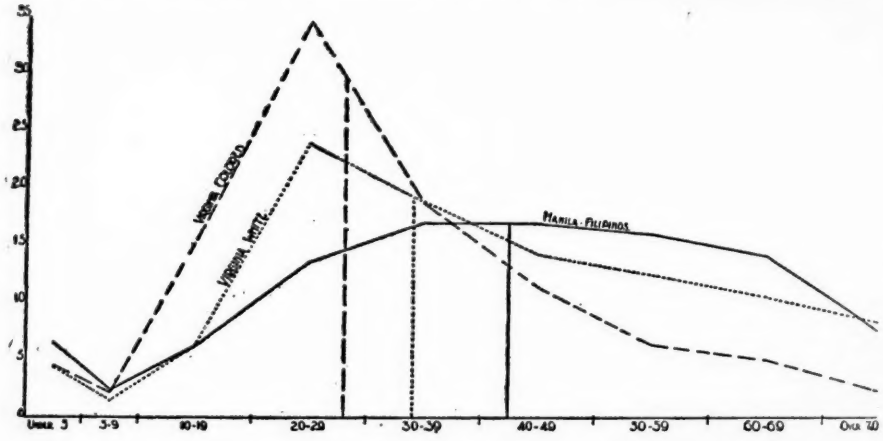

CHART 5. GRAPH SHOWING PERCENTAGE DISTRIBUTION OF TUBERCULOSIS DEATH RATES BY AGE GROUPS, FOR THE FILIPINO RESIDENTS OF MANILA, 1921–1922, THE WHITE POPULATION OF VIRGINIA, 1920, AND THE COLORED POPULATION OF VIRGINIA, 1920

The rates for Manila and for the colored population of Virginia are adjusted to the age distribution shown by the Virginia whites. The vertical lines indicate the median age at death in each group.

corresponding to the median age at death for the three races considered. These differ in a very interesting way. The median age at death from tuberculosis for the Virginia negro is slightly over twenty-eight years, that of the white Virginian, thirty-four years, and that of the Filipino forty-two and a half. This one figure appears to give a serviceable index of the degree of tuberculization of a group. The lower the resistance to the disease the lower the median age at death and *vice versa*. In spite of the high degree of racial resistance indicated for the Filipino, chart 5 shows that the percentage of deaths under ten, and especially under five, is higher than that shown for either of the other races.

According to the epidemiological criteria already cited, chart 5 should be interpreted as indicating the existence of conditions allowing a considerable proportion of children to be overwhelmed by massive doses of the virus before an effective resistance can be acquired.

The high death rates shown by the Filipino in the older age groups afford confirmation of the results of the physical survey of Filipino soldiers described later. The survey showed that the incidence of manifest lesions increased with advancing years, a condition the reverse of that found in the white soldier.

Thus, while the total and specific death rates show that the Filipino is constantly exposed to tuberculous infection, the age distribution shows that individually he possesses a very high degree of resistance to the ravages of the disease and is able to combat infection much longer than members of the white or colored race. The possibility that this relative immunity may be inherited, as well as that it may be the result of repeated small inoculations in early life, is strongly indicated by the work of Guyer and Smith (1923) on the inheritance of artificially induced immunity in rabbits.

The figures already studied seem to show conclusively that the high death rate from tuberculosis in the Philippines, in a race thoroughly tuberculized, and at the same time showing a high mortality in childhood, must be largely due to conditions permitting massive infection, which should be largely preventable. In confirmation of the inference on which the above conclusion is based, that the high death rates of the older age groups are significant of a large proportion of deaths from chronic fibroid phthisis, the deaths in each age group have been classified according to pathologico-anatomical type of the disease. Table 4 shows these relations for the Archipelago, 1918, table 5 for Manila, 1918–1923, and table 6 for the Registration States of the United States, 1920. It is seen that after very early childhood the rates for pulmonary tuberculosis are higher than those in the United States. This is particularly true in the age groups, 2 to 9, inclusive, and affords additional evidence that the more chronic pulmonary type of the disease predominates in the Filipino.

The low figures for pulmonary tuberculosis and meningitis, and the high figures for white swellings in the first age group, are open to suspicion as partially, at least, caused by erroneous diagnoses. It is difficult, however, to understand what nontuberculous condition in children under one year of age would be confused with joint tuberculosis. Most of

TABLE 4

*Tuberculosis, Philippine Islands, 1918**

Percentage distribution of pathological types by age*

	LESS THAN 1 YEAR	1 YEAR	2–4 YEARS	5–9 YEARS	10–19 YEARS	30–29 YEARS	30–39 YEARS	40–49 YEARS	50–59 YEARS	60–69 YEARS	70 + YEARS	TOTAL
Pulmonary tuberculosis	14.0	42.6	62.9	78.0	91.7	95.4	95.9	96.0	95.8	95.6	96.2	94.0
Acute miliary		0.6	0.67	0.83	0.74	0.38	0.35	0.42	0.27	0.27	0.14	0.36
Meningitis	12.8	17.7	0.4	5.9	0.68	0.13	0.08	0.01	0.1	0.07		0.5
Abdominal tuberculosis	1.7	2.9	4.7	2.9	1.6	1.2	1.1	1.07	0.98	1.25	1.16	1.25
Pott's disease	5.5	4.1	2.2	2.0	0.3	0.15	0.16	0.06	0.06	0.02	0.07	0.23
White swellings	30.2	8.2	6.5	4.8	1.8	0.55	0.49	0.38	0.54	0.61	0.42	0.97
Tuberculosis, other organs	34.0	18.9	8.5	2.7	1.4	0.83	0.82	0.73	0.98	0.81	0.62	1.33
Disseminated	1.7	4.7	4.9	2.7	1.5	1.3	1.1	1.17	1.16	1.35	1.35	1.36

* Census, Philippine Islands, Volume 2.

TABLE 5

*Tuberculosis in Manila, 1918–1923 inclusive**

Percentage distribution of pathological types by age

	LESS THAN 1 YEAR	1 YEAR	2–4 YEARS	5–9 YEARS	10–19 YEARS	20–29 YEARS	30–39 YEARS	40–49 YEARS	50–59 YEARS	60–69 YEARS	70 + YEARS	TOTAL
Pulmonary tuberculosis	24.5	28.3	46.5	57.7	87.0	93.0	94.5	93.8	93.0	93.0	92.0	88.0
Acute miliary	0.8	0.8	1.6	0.9	1.9	1.1	0.4	0.6	0.3			0.74
Meningitis	53.6	57.8	34.6	21.9	3.7	1.1	0.6	0.4	0.3			4.6
Abdominal tuberculosis	3.2	4.9	11.3	9.4	2.9	2.8	3.2	3.3	4.4	5.3	4.7	4.0
Pott's disease	0.8	0.4		0.3	0.26	0.4						0.09
White swellings		0.4	0.23	0.6	0.26	0.08						0.098
Tuberculosis, other organs	8.0	2.5	2.5	2.4	0.26	0.5	0.5	0.7	0.8		1.1	0.85
Disseminated	8.8	4.9	3.2	5.8	3.6	1.1	9.7	1.0	1.1	0.9	0.8	1.5

* Figures from the Annual Reports of the Philippine Health Service.

TABLE 6

*Tuberculosis deaths, United States Registration States, 1920**

Percentage distribution of pathological types by age

	LESS THAN 1 YEAR	1 YEAR	2–4 YEARS	5–9 YEARS	10–19 YEARS	20–29 YEARS	30–39 YEARS	40–49 YEARS	50–59 YEARS	60–69 YEARS	70 + YEARS	TOTAL
Pulmonary tuberculosis	33.3	29.9	26.9	40.0	80.5	89.6	91.0	90.4	90.0	90.0	88.3	85.0
Acute miliary	6.0	6.0	4.7	5.5	5.8	3.7	2.8	2.3	1.7	1.7	1.2	3.2
Meningitis	46.4	52.3	52.55	35.6	5.6	1.7	1.2	1.1	0.8	0.6	0.3	4.8
Abdominal tuberculosis	7.4	6.6	7.8	8.8	4.1	2.9	2.5	3.2	3.5	3.9	5.2	3.5
Pott's disease	1.1	1.1	3.6	3.8	1.4	0.6	0.72	0.9	1.1	0.9	0.9	0.9
White swellings	0.4	0.5	1.3	2.0	0.8	0.3	0.3	0.4	0.5	0.6	0.9	0.4
Tuberculosis, other organs	3.2	2.4	2.5	3.4	1.4	0.8	1.0	1.3	1.6	1.8	2.4	1.3
Disseminated	1.6	0.8	0.5	1.0	0.6	0.3	0.3	0.4	0.5	0.5	0.5	0.4

* Rate computed from the 1920 United States Census population figures and the 1920 Vital Statistics of the Census Bureau.

these cases were reported from the provinces, as the statistics for Manila, table 5, show a very low figure, there being only 10 such cases reported in the city in this age group during the six years, 1918 to 1923, inclusive.

White swellings in tables 5 and 6 show the usual distribution, with the high point of incidence at about the fifth year. The total rate for the whole islands is over twice as high as for the United States Registration Area and ten times that for Manila, where this lesion is surprisingly infrequent in a population with a much higher total death rate for tuberculosis. The etiology of bone tuberculosis has been the subject of a good deal of experimental work. It arises from the lodging of the bacilli in capillary endings and lymph spaces, where they are carried from some primary focus. Its normal period of greatest frequency is the fifth to fifteenth years, although it occurs at all ages.

Raw and Frazer (quoted by Calmette, 1923) collected data from Great Britain which tended to prove that bone infections were usually due to the bovine tubercle bacillus. While this might account for the low rates in Manila, it does not explain the higher rates in the provinces. Fresh cow's milk is a small factor in the diet of the Filipino infant and even the milk of the carabao is relatively little used. In general, this lesion is more frequent in the less highly tuberculized and, therefore, as would be expected, it is more frequent in rural populations less thoroughly exposed to tuberculous infection at the ages of greatest frequency of this lesion than are the residents in urban communities.

Tuberculosis of the vertebrae shows lower rates in the Philippines than in the Registration Area, and, as in the case of white swellings, Manila is very low and the provinces furnish most of the cases.

The figures from the hospital clinics do not show a very large number of bone and joint cases, and it seems to be the general opinion that, considering the total incidence of tuberculous infections, lesions of these structures are surprisingly infrequent.

Acute miliary tuberculosis presents a typical series of rates, although the whole is considerably lower than those for the Registration Area. Part of this, again, is due to the difficulties of diagnosis.

Disseminated tuberculosis runs high in the statistics of Manila and in the Philippines as a whole. A number of these cases in the upper age groups should probably be otherwise classified, as this form of the disease is usually primary in those with very little or no immunity before the fatal dose was received. I have not seen it in others than children, and believe that the fairly rapid cases of pulmonary tuberculosis in

youth, with terminal extensions to the intestines and other organs, have been classified as disseminated tuberculosis and account for the relatively high rates for this condition.

Abdominal tuberculosis, aside from terminal extensions, means exposure to large doses before immunization is complete.

Abdominal tuberculosis is not as confusing to diagnose as the more obscure miliary and disseminated types, and the Philippine figures may be taken with less reserve. The rates for this disease are higher for Manila and lower for the Philippines than are those of the Registration Area. The existence of a considerable number of such cases in Manila also indicates that this condition arises from the ingestion of bacilli from human sources as readily as from the bovine strain.

Tuberculous meningitis shows a higher incidence in both Philippine and comparison tables than its actual occurrence as a primary condition. In fact, it probably is never primary, but is usually part of a general miliary process in which the brain is involved and the symptoms from that organ dominate the clinical picture. The figures have no value as a basis for determining the picture of tuberculosis. Unless a part of an acute process, these cases are due to accidental tuberculous involvement of a vessel.

HOSPITAL STATISTICS

Table 7 is compiled from the annual reports of the Philippine General Hospital (1922 and 1923) and may be compared with table 11 compiled from autopsy reports, the larger proportion of which were of deaths in that hospital.

Pulmonary tuberculosis is low in the hospitalized cases, as the Philippine Health Service Hospital, San Lazaro, is supposed to handle all cases of this condition requiring hospitalization, with the less advanced cases going to a tuberculosis sanatorium. The ratio in the out-patient clinic is more nearly representative of the ratios present in the general community.

It is evident from the percentages shown that more attention is paid to tuberculosis in the Manila hospital than in the Cebu branch, especially in the Out-Patient Department. The hospital percentages are not at such great variance (5.75 and 5.95 to 4.26 and 3.98), but the out-patient percentages, 5.65 and 0.79, are so distinctly different that we must infer that little attention is paid to this condition in the Cebu dispensary.

The rate for abdominal tuberculosis is high in the hospitalized cases,

TABLE 7

Tuberculosis admissions to the Philippine General Hospital by clinical types

	IN-PATIENTS								DISPENSARY			
	1922				1921				1921			
	Manila		Cebu		Manila		Cebu		Manila		Cebu	
	Ratio	Total	Ratio	Total	Ratio	Total	Ratio	Total	Ratio	Total	Ratio	Total
Total admissions		10,088		1,836		9,489		1,766		46,948		33,965
Tuberculosis (all)		581		78		563		70		2,646		267
Percentage of total		5.81		4.26		5.95		3.98		5.65		0.79
Tuberculosis												
Pulmonary	69.2	402	68.0	53	72.0	405	60.0	42	86.5	2,285	73.6	197
Acute miliary		3				3		3				2
Meningitis		15				14				1		1
Abdominal												
Intestinal	6.2	13	15.4	12	8.2	13	17.1	12	1.27	17		1
Peritoneal		23				32				17		
Vertebrae	7.2	21		1	3.9	10		2	2.75	14		7
Bones and joints		21		1		12				43		
Lymph nodes												
Pulmonary	10.85	62			9.25	2			7.15	189		
Others		1				50						
Other organs		20		11		19		11		80		57
Disseminated						2						2

Note: Cases of manifest pulmonary tuberculosis are usually sent to San Lazaro Hospital, and are not intended for hospitalization at the Philippine General Hospital.

as this condition naturally requires bed treatment. This manifestation of the disease, together with the appreciable number of lymph-node infections, gives a good idea of the frequency of exposure to relatively large doses of the virus.

This group of figures is the only one we have been able to obtain that shows the incidence of bone and joint tuberculosis in the living. There are often conditions of chronic bone infection which are confusing, and many of the diagnoses have not been confirmed by accurate laboratory methods. The doubtful cases have been excluded from the table, in so far as was possible from the study of the diagnoses, and it is felt that the ratios are sufficiently close to be representative of the actual ratios present in Manila. The bone lesions of yaws may occasionally be confused with those due to tuberculosis, particularly in dispensary patients on whom little determinative laboratory work is done. Medical men, who serve the more prosperous group of Manila Filipinos, state that bone tuberculosis is very rare in their experience, although they have no definite figures.

In the pediatrics clinic and wards there were no evidences of any large proportion of tuberculous infections of childhood. The age group is from one to ten years, and tables 8 and 9 show the incidence. The percentage of autopsies obtained from the pediatrics wards is not high. These tables should be compared with the autopsy table 10. By grouping, in tables 8 and 9, we obtain comparative ratios, though from small numbers.

Bone and joint tuberculosis seems rare, considering the other evidences of massive infection, and this is true generally in the Philippines. The pigmented skin of the Filipino renders him relatively insusceptible to sunburn. He wears little clothing, particularly in childhood, so that sunlight, considered so beneficial to bone tuberculosis, has free access to his body. It is possible that this exposure to abundant tropical sunshine has a determining influence on the incidence of such lesions.

Abdominal tuberculosis has a considerable incidence in the hospitalized cases in children, though not as high as in the total hospital cases in the general table.

Lymph-node tuberculosis is seen to be high in all groups, as would naturally be expected.

The high rates for abdominal tuberculosis in the Cebu clinic are of interest, more particularly as the total death rate is below the average of the provinces and as the incidence in the Army survey showed a rela-

TABLE 8

Tuberculosis in pediatrics clinic, Philippine General Hospital, Manila

ALL	1918							1921		
	NEW CASES	GROUP	PERCENTAGE OF TOTAL TUBERCULOSIS CASES	SECOND VISITS	TOTAL	GROUP	PERCENTAGE OF TOTAL TUBERCULOSIS CASES	TOTAL	GROUP	PERCENTAGE OF TOTAL TUBERCULOSIS CASES
All diseases	6,363			6,138	12,501			10,280		
Tuberculosis of the lungs										
Bronchitis, tuberculous	2				2			19	(Peribronch)	
Bronchopneumonia, tuberculous	1				1			2		
Tuberculosis, lungs	79			29	108			114		
Tuberculosis, lung, advanced	1				1			1		
Tuberculosis, lung, incipient	13	104	86.0	15	28	160	88.0	16	153	92.7
Tuberculosis, lung, mod. advanced	5			3	8			1		
Tuberculosis, lung, with bronchitis	2			1	3					
Tuberculous pleurisy (postoperative)	1			8	9					
Pott's disease										
Spondylitis, tuberculous	1	1	0.8	3	4	4	2.2			
White swellings										
Arthritis, tuberculous	1	2	1.6		1	2	1.1			
Tuberculosis, hip joint, left	1				1					
Tuberculosis of other organs										
Adenitis, tuberculous	7			1	8			9	9	5.3
Adenitis, tuberculous, axilla	1				1					
Adenitis, tuberculous, cervical	4	14	11.6	5	9	20	11.0			
Adenitis, tuberculous, peribronchial	1				1					
Lymphadenitis, tuberculous, cervical	1				1					
Abdominal								3	3	1.8
Total		121				182			165	
Percentage of total cases due to tuberculosis			1.9				1.4			1.6

tively low figure. This renders the death rates open to suspicion, for we expect a low incidence rate in men selected for military service, but a higher tuberculosis death rate in the general population. A high death rate from abdominal tuberculosis is an indication of massive infection or an imperfectly tuberculized population.

TABLE 9

Pediatrics cases in hospital

	1918			1921		
	Total	Group	Percentage of total tuberculosis cases	Total	Group	Percentage of total tuberculosis cases
All diseases	1,150			1,051		
Tuberculosis of the lungs						
Tuberculosis, peribronchial	19	48	67.5	10	25	73.5
Tuberculosis, pulmonary	29			15		
Tuberculous meningitis	14	14	19.7	4	4	11.8
Abdominal tuberculosis						
Tuberculosis, intestinal	1	3	4.2	1	2	5.9
Tuberculosis, peritoneal	2			1		
Abscess cold (bone)	2	2	2.8	1	1	2.9
Adenitis	4	4	5.6	1	2	5.9
Scrofula				1		
Total tuberculosis		71			34	
Percentage of total cases due to tuberculosis			6.2			3.2

SUMMARY

A study is presented, based on the annual reports of the Philippine General Hospital and on the incidence rates for the anatomical types of the disease made from these reports.

The incidence tables show a preponderance of the chronic tuberculosis of a well-tuberculized community, while the considerable numbers of cases of abdominal and lymph-node tuberculosis indicate overexposure of the population to tuberculous material.

A higher rate for abdominal tubercolosis is shown for Cebu than for Manila. This high rate is not readily explained.

AUTOPSY DATA

A study of the tuberculous lesions found in 10,000 autopsies,[5] table 10, is compiled from those autopsy protocols from 10,000 autopsies in which the lesions were described with such definiteness that the data could be included in a table.

The conditions which modify to some extent the conclusions to be drawn from table 10 are somewhat peculiar, and a brief statement concerning them may be found of value. In the first place, chronic pulmonary tuberculosis is not a cause for admission to the Philippine General Hospital, from which the majority of these autopsies are derived. The Philippine Health Service Hospital at San Lazaro is intended for such cases, when in need of hospitalization, while there is a tuberculosis sanatorium and several clinics for treatment of earlier cases of this disease. Furthermore, it has not been the custom to request autopsies on deaths manifestly due to pulmonary tuberculosis. Not all of these cases died of their tuberculosis. On the other hand, no particular search was made for tuberculous lesions. The ones recorded had manifest pathological changes in the majority of cases, either considered as causing or contributing to decease, and therefore do not adequately indicate the incidence of the tuberculous lesions in the 10,000 autopsies. In the year and a half in which the writer has been studying this disease in the Philippines, approximately 200 autopsies have been seen. Except in children of the lower ages, no case has been seen in which there was absence of some lesion which could be determined to be one of tuberculosis. It should be stated, however, that the autopsies in Manila do not include members of the tribes residing in the hills and relatively remote from civilized communities. The deaths are from people who lived in an urban community or its immediate surroundings, and who therefore had, for the greater part of their lives, been in contact with tuberculous material.

The preponderance of chronic pulmonary tuberculosis and the ratios according to age are the same as are found in any tuberculized community.

The column on acute general miliary tuberculosis was the most difficult one to compile, as the existence of old lesions had to be searched for carefully in each autopsy protocol. It will be noted that it continues throughout the first three decades and shows no particular increase

[5] From a tabular study of 10,000 autopsy protocols made by Dr. Regino J. Navarro, of the Department of Pathology, School of Medicine, University of the Philippines.

TABLE 10

Incidence by age of anatomical types of tuberculosis in 1,057 autopsies in which tuberculous lesions were recorded. Selected from 10,000 protocols

AGE GROUP	CHRONIC PRIMARY TUBERCULOSIS	ACUTE GENERAL MILIARY	LYMPH NODES	LYMPH NODES PLUS PULMONARY	LYMPH NODES, TOTAL (3 + 4)	ABDOMINAL, PERITONEAL ALONE	PERITONEAL AND INTESTINE	TOTAL ABDOMINAL (6 + 7)	PRIMARY, ACUTE GENERALIZED	ACUTE, LUNGS ONLY	ACUTE, TOTAL (9 + 10)	MENINGITIS ALONE	KIDNEY, LIVER AND SPLEEN	TOTAL	CHRONIC FIBROUS PLEURISY
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Less than 1 year		6	2		2	1	1	2	10	4	14			24	2
1–4 years		17	8	1	9	4	6	10	44	9	53	1		90	15
5–9 years	2	3	4	1	5	4	1	5	9	3	12	4	1	32	19
10–19 years	40	17	3	2	5	4	1	5	3		3	2	1	73	44
20–29 years	202	9	6	3	9	4	5	9					2	231	94
30–39 years	199	9	5		5	1	3	4					3	220	84
40–49 years	140	2				2	7	9					5	156	53
50–59 years	91	1				1	3	4				1	1	98	26
60–69 years	71	2	2		2	2		2					1	78	23
70–79 years	34	2												36	14
80–89 years	11	2				2		2						15	6
90 years, and over	3					1		1						4	2
Total	793	70	30	7	37	26	27	53	66	16	82	8	14	1,057	382
Percentages	75.0	6.6	2.8	0.66	3.46	2.46	2.55	5.0	6.6	1.5	8.1	7.6	1.3		

with later age groups. It is probably the least accurate of any of the columns in this table.

Lymph-node tuberculosis shows the characteristic distribution which one would expect in a community of which there is a large amount of active disease and a lack of that hygiene within the community which should lower the incidence of this type. It is, however, characteristically large in the early age groups.

Tuberculosis of the peritoneum and intestines also shows relatively high figures which are confirmatory in the findings in the lymph-node group. Milk, except canned milk, is not a part of the diet to any appreciable extent among Filipinos. We believe with Calmette (1923) and others, however, that the intestinal tract offers a method of entrance for the tubercle bacilli which can give rise to intestinal and peritoneal or pulmonary lesions, or both. The large amount of infectious material, with which most Filipinos come in contact in the unsanitary surroundings of their homes, is undoubtedly responsible for the ingestion of large amounts of tuberculous material from human sources. The large amount of peritoneal and intestinal tuberculosis in Filipinos is strong evidence that tuberculous infection can arise from bacilli ingested with food contaminated with the tubercle bacillus and not originally containing it, as there is little or no possibility of infection from milk in these Islands.

Wieting, as quoted by Bushnell (1920), calls attention to the high rate of abdominal tuberculosis in Turkey. In this population he states that milk, butter, cheese and meat play no rôle of importance in the diet. He was dealing with a poorly immunized group at that time, as he states that bone and joint tuberculosis constituted 54 per cent of surgical tuberculosis and 11.5 per cent of all surgical cases.

The columns *Primary Tuberculosis* are compiled after careful study of the autopsy protocols, and the age distribution found is likewise characteristic of the conditions in a thoroughly tuberculized community, in which little effort at protection of the young from infectious material is made. The lesions were of acute fulminating type, with massive consolidations in the lungs or large caseous dissemination throughout the organs of the body, with many lymph-node groups involved in all except those cases which appear to be confined to the lungs, and in some of these distant lymph-node groups were frequently involved.

In column 12 it will be noted that few cases were recorded as due to meningitis alone. This disease is, of course, secondary to an infection of other parts of the body, but in the cases reported careful study of the

autopsy protocols failed to reveal any such lesion. It is believed, however, that such a one did exist and was overlooked, because no definite search was made for it by the pathologist.

The same statement might also be made with reference to column 13 (kidney, liver and spleen), although it is possible that the liver might be the site of tuberculosis from ingestion of infected material.

Chronic fibrous pleurisy was not included in the tabulation, but forms column 15 beyond the total. It occurs so much more frequently than is recorded in this series that it simply indicates the number of times that the pathologists recorded this lesion and has no bearing on its incidence or on the incidence of tuberculosis. There were a few cases recorded in which a definite tuberculous empyema was found, and a few in which serous pleurisy with miliary tubercles on the pleura was noted, but in those instances the important condition was a chronic pulmonary tuberculosis or acute tuberculosis of the lungs in the primary group, and the pleural lesion had no significance from an epidemiological standpoint.

Table 10 is, we believe, representative of the distribution of the anatomical types of lesions in Filipinos in the well-tuberculized community of Manila, and is probably fairly representative of the deaths in civilized Filipinos throughout the Islands, although, as we repeat for emphasis, it does not adequately represent the total incidence of tuberculous lesions.

The autopsy figures for Manila also showed the distinctly higher figures one expects in miliary tuberculosis by reason of accurate diagnoses, and the rate for intestine and peritoneal involvement is distinctly higher than that of the general statistics for the same reason.

No bone lesions are recorded, and apparently did not exist in this group. Bone involvements are relatively rare, and there are other conditions with which they may be confused. Hospital admissions, dispensary cases and the Health Service Reports must be consulted to study the incidence of these lesions.

The following summary of organs involved in 80 cases, with tuberculous involvement found in 500 autopsies in children from birth to five years, is instructive, although the case incidence was not given by the author, Mendoza-Guazon (1917):

Total cases	80
Lungs	58
Lymph node groups	133
Meninges	14 (Brain, 7)
Pleura	54
Peritoneum	11
Gastrointestinal	54
Bones and joints	2

The involvements are typical of tuberculosis at these ages anywhere, and in the absence of any data from which rates might be calculated do not call for further comment.

The following quotation from Calmette (1923) is of interest in this connection:

As I have already said, we are particularly indebted to the work of Landouzy (1886–1891), and to that of Baginsky, of Comby, of Hamburger and Sluka, of Emmet Holt, Kuss, Hutinel, and others, for our knowledge of the fact that, far from being rare in the first months of life, as was previously thought, tuberculosis is so frequent in very young infants that it is encountered in about one-third of the autopsies of babies under two years.

In the course of the first year, according to Landouzy, 27.8 per cent and, during the second year, 16.2 per cent of deaths are due to this disease.

According to H. Barbier and Bondon, out of each 1000 children from birth to 15 years, 392 die, and of these 116, or 29.2 per cent, succumb to tuberculosis. This mortality is apportioned as follows

	TOTAL MORTALITY		ACTUAL MINIMUM MORTALITY FROM TUBERCULOSIS	REMAINING ALIVE AT THE END OF THE YEAR
	Per cent	Number of children		
From birth to 1 year	20.0	200	30 (to 60)	800
1 to 2 years	6.5	48	12	752
2 to 3 years	3.5	26	11	726
3 to 4 years	2.3	16	7	710
4 to 5 years	1.6	12	7	698
5 to 10 years	8.4	56	33 (in 5 years or 6.5 per year)	642
10 to 15 years	5.5	34	16 (in 6 years or 2.6 per year)	608

These figures should be regarded as approximately accurate, but if anything rather below the actual state of affairs, since we know that many tuberculous lesions are not found, even though sought for with the utmost care at autopsy. And further it must be remembered that a very large number of children, who died outside of hospitals without the diagnosis being established by autopsy, have in reality died as a result of tuberculous infection.

It is likewise a fact well known among clinicians that in the infant, particularly up to the age of 6 months, tuberculosis is almost always of extremely grave prognosis. Unfortunately, it intervenes also with great frequency as a fatal complication at the end of other illnesses which in themselves are not serious.

I shall borrow from an article by Leon Bernard the following table which summarizes the principal statistics published in France and in Germany:

Percentage of childhood mortality from tuberculosis

	HUTINEL	KUSS	COMBY	MANTOUX	KOSSEL	HAMBURGER	BINSWANGER
From birth to 3 months	3.5	1.16	2		1.6	6	2.2
From 3 to 6 months		13	18	7	11	17	8.4
From 6 to 12 months			27	16		22	16.8
Second year	33	24	43	23			
2 to 4 years		50				30	
Puberty						53	

These tables may be compared with our tables 3, 4, and 5, and serve as a check on the rates in the early age groups. It is evident that if these figures are correct ours are too low in the lowest age groups but reach nearer the proper level in the 10–19 year period.

SUMMARY

A study of the incidence of the anatomical types of tuberculous lesions in 10,000 autopsies in Filipinos is reported. The incidence of these types by age is recorded in a table (table 10), the rates of which indicate that Manila is a well-tuberculized community in which hygiene and sanitation are as yet far from ideal, at least in so far as tuberculosis is concerned.

The rather high rate for abdominal and intestinal tuberculosis indicates infection through the intestinal tract by tuberculous material from human sources, there being little opportunity for infection from animals.

PHYSICAL SURVEY OF FILIPINO AND AMERICAN SOLDIERS

The relatively high incidence of pulmonary tuberculosis, as a cause for discharge for disability of Filipino soldiers, has been of particular interest during the period in which native troops have formed a part of the United States Army. Table 11 shows the discharge rates for white, colored and Filipino soldiers for twenty years (Callender, Shepard and Kirkwood, 1925).

The increase in discharge rates, 1918–1920, is dependent on the same factors which in the same years caused a sharp rise in the death rates and which have been discussed in the section devoted to the mortality rates.

There is, however, another factor which enters here, namely, the demobilization of 1918, which resulted in decreasing the average strength on which the rate is based before the discharges were accomplished. This, by producing a smaller divisor, naturally increased the rates.

Methods of examination: The soldiers reported to the place for examination, where cards were made out. They were then weighed, had their blood-pressure taken, and reported to the examiner stripped. A general

TABLE 11

Discharge rates per 1,000 per annum for disability from tuberculosis, U. S. Army, 1903–1922, inclusive

YEAR	UNITED STATES		FILIPINO
	White	Colored	
1903	2.66	1.89	5.01
1904	1.72		2.60
1905	2.50	3.00	2.02
1906	2.93	3.88	4.76
1907	2.88	5.37	4.49
1908	2.32	2.83	3.84
1909	2.04	3.95	5.06
1910	3.88	2.68	2.64
1911	2.67	2.00	2.09
1912	1.45	0.74	4.01
1913	1.99	3.24	7.07
1914	1.88	2.74	4.38
1915	1.62	2.75	4.54
1916	1.87	5.75	2.87
1917	5.57	8.57	1.45
1918	3.56	4.04	18.56
1919	6.38	6.06	17.76
1920	13.21	22.74	10.01
1921	2.55	3.58	5.00
1922	1.59	2.40	6.26
Averages.....	3.26	4.64	5.72

physical examination was made, as well as the special examination of the chest. All with suspicious signs were referred to the X-ray department, where the three members of the examining board examined the plates and recorded the findings, coördinating the results of the X-ray and physical examinations.

Only those cases were called positive in which positive physical signs were confirmed by the presence of characteristic shadows in the paren-

chyma of the lungs by stereoscopic roentgenogram. For the purpose of diagnosis alone, cases showing definite moisture in addition to the above were considered active. It was the intention to order the "active" cases to the hospital for observation, and to determine there whether clinical activity as indicated by temperature, loss of weight, etc., was really

TABLE 12

Incidence of tuberculous lesions in the lungs, according to age in American troops (white)

AGE	ABSOLUTE NUMBERS			PER CENT POSITIVE		TOTAL PER CENT POSITIVE
	Negative	Inactive	Active	Inactive	Active	
19 years	152	11	6	6.5	3.5	10.0
20–24 years	548	45	10	7.2	1.7	8.9
25–29 years	249	15	2	5.6	1.2	6.8
30–34 years	166	2	1	1.2	0.6	1.8
35—39 years	97	5	1	5.1	1.1	6.2
40 years, and over	90	5		5.5		5.5
Total	1,302	83	20	5.8	1.5	7.3

TABLE 13

Incidence, absolute numbers and ratios (per cent) of manifest tuberculosis by age groups among Filipino troops

AGE	ABSOLUTE NUMBERS			PER CENT POSITIVE
	Negative	Inactive	Active	
19 years	130	12	3	10.3
20–24 years	919	145	36	16.4
25–29 years	562	78	24	15.4
30–34 years	289	58	14	20.0
35–39 years	226	55	16	23.9
40 years, and over	130	31	13	25.1
Total	2,256	379	106	17.7
Total absolute numbers	2,741			

present, or whether such signs of moisture as were found were due to other causes, such as pleural crepitations and signs of moisture around a clinically quiescent lesion. This programme was followed out in the survey on the American troops (table 12) and in 64 of the 106 in the Filipino troops in table 13.

Table 12 shows the age distribution in the American troops. It will

be noted that there is a definite decrease of tuberculosis as the age of the group increases. This, we believe, should be attributed to the weeding out during service of those cases which, because of large infections, break

TABLE 14

Incidence, absolute numbers and ratios (per cent) of tuberculous lesions of the lungs compared with provincial densities and death rates

PROVINCE	ABSOLUTE NUMBERS				PER CENT POSITIVE	PROVINCIAL DENSITY PER SQUARE MILE (1918)	DEATH RATES PER 100,000 (1918)
	Total	Negative	Inactive	Active			
Abra	88	69	16	3	21.8	49	274
Camarines (Bicol)	288	248	32	8	13.9	156	378
Bataan and Zambales	12	9	3		33.3	75	473
Batangas	22	17	5		22.8	268	293
Bohol	37	33	2	2	10.8	233	237
Bulacan	20	18	2		10.0	248	515
Cagayan	103	87	13	3	15.5	63	292
Cavite	20	17	2	1	15.0	339	279
Ilocos Norte	296	252	36	8	14.9	169	264
Ilocos Sur	144	114	22	8	20.8	492	245
Isabela	20	17	3		15.0	28	280
Laguna	23	18	3	2	21.7	271	317
La Union	151	126	20	5	16.5	459	256
Leyte	198	167	22	9	15.7	199	319
Masbate, Mindoro, Marinduque, and Romblom	8	7	1		12.5		
Mindanao	40	31	9		22.2		
Mountain	11	9	2		18.2		
Nueva Ecija	42	32	7	3	23.9	110	294
Nueva Viscaya	27	21	3	3	22.2	10	145
Negros	96	82	12	2	14.6	125	340
Pampanga	183	125	45	13	31.7	313	427
Pangasinan	184	152	26	6	17.4	291	384
Panay	372	312	47	13	16.1	200	438
Rizal	45	36	7	2	20.0	256	614*
Samar	78	68	7	3	12.8	72	123
Tarlac	48	42	5	1	12.5	146	356
Tayabas	23	16	5	2	30.4	65	456
Cebu	147	121	19	7	17.7	458	233
No province	15	10	3	2	33.3		
Total	2,741	2,256	379	106	17.7	90	335

* Manila deaths included.

down and are discharged. The corresponding figures for Filipino soldiers are shown in table 13. It is seen here that the incidence of discoverable tuberculosis increases with age.

In table 14 there is seen to be a marked variation in the rates of the provinces, which is, in part, due to the small size of some of the groups.

In table 15 certain of these groups have been combined, as is indicated in the table, to obtain larger figures for computation of rates.

The most noticeable rates in table 14 are the high rates for Pampanga, 31.0 per cent, and the wide difference between the two Ilocos provinces,—

TABLE 15

Combined table of provincial groups

PROVINCIAL GROUPS	TOTAL	NEGATIVE	POSITIVE		PER CENT POSITIVE
			Inactive	Active	
I. Abra, Ilocos Norte, Ilocos Sur, La Union, Cagayan, and Isabela	802	665	110	27	17.1
II. Cebu, Samar, Negros, Leyte, Bohol, and Panay	928	783	109	36	15.6
III. Pampanga	183	125	45	13	31.7
IV. Pangasinan	184	152	26	6	17.4
V. Mindanao, Bataan, Zambales, Mindoro, Marinduque, Masbate, Romblom, Mountain, Neuva Ecija, Tarlac, and Neuva Viscaya	188	151	30	7	19.7
VI. Batangas, Bulacan, Cavite, Laguna, Rizal, Tayabas, and no province	168	132	27	9	21.4
VII. Camarines, Sorsogon, and Albay	288	248	32	8	13.9
Grand total	2,741	2,256	379	106	17.7

Ilocos Sur, 20.0 per cent, and Ilocos Norte, 14.9 per cent. Comparing the rates for Pampanga, we find a relatively high population density and a similarly high death rate. On the other hand, in the Ilocos provinces, although we have the highest density of population of the Islands, the mortality is not higher than in many other provinces. Naturally, mortality rates by provinces are less dependable than those from the entire Islands, but a high incidence of manifest lesions from an area with high population density and a low mortality, or one below the same relative position in the mortality table, is to be expected if our surmise

is correct that the deaths from tuberculosis in childhood are not recorded as such. High density means high infection rate, many tuberculosis deaths in early life, and a large number of recognizable lesions in the survivors, as we have suggested in our discussion of the mortality statistics.

Malaria is a factor in the Ilocos provinces, but it has not the bad reputation there that it has in Pampanga. In the latter province we cannot explain this high incidence on any such basis as in Ilocos Norte and Sur.

Pampanga has a high malaria rate. The country is low and swampy, and the population is for the most part in relatively crowded barrios and municipalities.

While we realize that the inheritance of an acquired immunity to infectious diseases, as a whole, is not well demonstrated scientifically, yet considerable evidence has been offered that such is the case. I refer particularly to the work, already mentioned, of Professor M. F. Guyer, of the University of Wisconsin (1923), by which he established that an immunity to typhoid bacilli in certain laboratory animals was transmitted by the mother to the offspring.

The above statement introduces the assertion of Professor H. Otley Byer, of the Department of Anthropology, University of the Philippines, that from 1600 to 1800 the Spanish brought troops from the native levies in Mexico to the Philippines, and, not having room for them in their boats, heavily laden for the return trip, started a colony in Pampanga, and added considerably to it from time to time, so that this imported strain is present in the Pampanga group alone. While it is extremely doubtful that this has had any influence, it is interesting, since the Mexican stock, mixed Aztec and Indian in origin, has, so far as we know, been exposed to tuberculosis only since 1500, while the Chinese probably have been in contact with the Philippines for 2000 years and were probably well tuberculized at the time. The Filipinos as a whole have probably been in contact with tuberculosis fully as long as most of the racial stocks which make up the white population of the United States. The gradual increase with age shows the opposite of the trend in the American troops. This corresponds to the findings in the mortality statistics where reference to it was made (see chart 5).

The total incidence in the two groups, 7.3 and 17.7, gives us the comparative rate of the two groups examined, there being 2.4 times as many positive cases in Filipinos as in Americans. This corresponds to some

extent with the difference in the death rates of the two peoples for 1920, 114.2 per 100,000 for Americans, and 250.0 for Filipinos, and would probably correspond still more closely if proportionately as many deaths in the early age group were reported from the Philippines as in the United States.

The applicants for enlistment examined by the same board gave distinctly higher percentages, but we must remember that prior to this series, recruits were examined by men not expert in the diagnosis of tuberculous lesions. Only the obvious cases were found by the average examiner, and the difference between the findings of ordinary examiners and experts is represented by the percentage of positives, 10.3, found in the first age group by the examiners of this survey, both experts in tuberculosis examination.[6] Pampanga showed the highest provincial rate here as in the troop survey series. I would estimate that about the same difference would exist in American applicants if examined, namely, that the examiners of this board would find about 12.5 positive in such applicants. For men of the age period, 17 to 21, the figures 12.5 for Americans and 30.0 for Filipinos are more representative than those for soldiers. It is not possible to estimate the number of tuberculosis cases in the general population from these figures of a small group.

In the Framingham experiment (City of Boston, Health Department, 1924) the conclusion was drawn that 1 per cent of the population had active pulmonary tuberculosis recognizable on physical examination, that over 1 per cent more had arrested cases of the disease likewise recognizable, and that under usual conditions in a Massachusetts city or town the number of active cases of pulmonary tuberculosis may safely be estimated to be nine or ten times the number of deaths reported annually as due to the disease; also that actual deaths from tuberculosis may be estimated to be 22 per cent more than reported deaths from the disease.

It is evident that these rates would not obtain here. Our findings show 3.7 active cases in the group examined, but the total is over four times the actives. Instead of 5 per cent positive cases, which would result in the Philippines by using the Framingham rates, it is quite probable that our figures should be above, rather than below, those found for the military group, as in Filipinos the lesions increase with age and are undeniably higher than those reported in the first decade.

[6] Major C. A. Shepard, M. C., and Captain R. C. Kirkwood, M.C., U. S. Army.

DISCUSSION

While recognizing the practical impossibility of the collection of accurate vital statistics in a country such as the Philippine Islands in their present stage of development, we have thought it worth while to present the published figures on the mortality from tuberculosis in order to give an idea of the magnitude of the tuberculosis problem in the Archipelago. The figures as given should be regarded as below, rather than greater than actual conditions for the reasons already given. The statistics for the City of Manila we regard as sufficiently accurate for use in analysis. The general correspondence between the Manila rates and those for the Islands as a whole, in the variations of the specific rates by age groups and in the relative proportions of pathologico-anatomical types of the disease, is sufficiently striking to suggest that they represent the conditions accurately.

The analysis of the mortality statistics, and the hospital and autopsy records given above agree with the physical survey of Filipino troops and lead to the conclusions stated below.

The high proportion of pulmonary tuberculosis, as shown in the classification of types, and as clearly indicated by the relatively high tuberculosis mortality in the older age groups, indicates beyond question that tuberculosis is no new disease among the Filipinos, but that the disease has doubtless been present for centuries and its presence has resulted in the development of that degree of tolerance, or ready acquirement of immunity, which is spoken of as tuberculization in a race. Under satisfactory hygienic conditions such a race should show a relatively low total death rate from tuberculosis, and especially the rates from the more acute types, characteristically occurring in childhood and early life should be low. The figures given show not only a high total rate, but a relatively high rate for infancy and childhood, as compared with the population of the United States. The total rates for Manila Filipinos, in spite of their tuberculization, are much higher than those of the colored population of Richmond. The colored race has only recently been in contact with tuberculosis and has acquired little resistance. The hygienic conditions of the city negro in the United States leave much to be desired. The only inference that can be drawn from the higher death rates in the relatively resistant Filipino is that his environment is such as to lead to infection with large doses of virus with excessive frequency, or that the conditions under which he lives reduce his resistance. The average

ration of the Filipino, while perhaps not inadequate to his needs, undoubtedly could be improved, from the standpoint of tuberculosis prevention, by the inclusion of more protein. A discussion of the Filipino dietary is outside the scope of this article. Malaria is doubtless a predisposing cause in many districts. The very general infestation of the population by various intestinal parasites also doubtless tends to reduce vitality to some extent, especially in certain districts. The prevalence of this condition, however, is the result of the same type of conditions, overcrowding and ignorance, or neglect of elementary hygienic principles, which also favor the ready spread of tuberculous infection.

The statistical evidence exists, then, to indicate that such poor hygienic conditions do exist among the mass of the natives of the Islands. At first glance it would appear that the almost universal *nipa* hut, without windows, and almost impossible to close completely, was a nearly ideal residence from the standpoint of ventilation. That even such airy dwellings may be dangerous is indicated by the figures showing the numbers living under each roof. Dr. Jose P. Bantug (Census Office, 1921, p. 1079) in the *Section on Vital Statistics* of the *Report of the Census of the Philippine Islands, 1918*, states that the average number of persons per dwelling had been found to be 5.5. "Likewise, 64 per cent of the houses consisted mainly of two rooms, with an average floor area of 20 square metres, which, with a height of two and one-half metres, would give each person an air space of less than 10 cubic metres. . . . . In case of illness in the family, especially if it be of an infectious nature, the proper isolation of the sick cannot very well be carried out." Dr. Bantug quotes Justice Villamor, as commenting on "the promiscuous life of the poorer classes in low and narrow dwellings, where parents, brothers and sisters sleep together with friends and visitors on the same floor and in the same room." Bantug further says (Census Office, 1921, p. 1084):

> The tendency to overcrowding in sleeping quarters is very common. Chiefly on account of the unreasonable fear of the night air, and in part to avoid the incursions of occasional thieves, the windows are kept tightly closed at night. It is hard to shut out all ventilation from the ordinary nipa houses, but with the windows shut, the doors closed, and the family *petate*[7] laid on the floor, the entrance of air is limited to the space between the eaves and the wall, to small crevices and to the uncovered portions of the floor. With the fresh air practically shut out, the atmosphere is further vitiated by the smoky kerosene

[7] *Petate*, a closely woven sleeping mat of vegetable fibre.

lamp, that poor substitute of the traditional *tinghoy* (oil lamp), kept burning all night long. And this is not all. When he feels chilly or when mosquitoes hover about his head, the poor laborer covers himself with a sheet from head to foot.

The ordinary man's conception of tuberculosis is that it is hereditary, and he has some curious notions and dangerous practises that favor its transmissibility. When sick he will not consult a physician because it is only a common cold, and he would not pay a fee for such a trifling ailment. While the disease is progressing the patient goes the usual round of his daily occupations. Household furniture and eating utensils are used by the family in common. At best there are but one or two drinking glasses, when the classical *saro*,[8] or the more common *lumbó*[9] is not in evidence. Promiscuous spitting is a universal habit. Dry sweeping, with all its evil consequences, is practised at all times. The open fireplace fills the living room with smoke. With these conditions and these practices is it a wonder that quite a few of the many persons exposed to the contagion contract the disease?

We need seek no further, then, for the explanation of the failure of Philippine tuberculosis mortality rates to decline, as seems to be the case in general elsewhere. Any great reduction in the tuberculosis death rate is not to be expected until the contagious nature of the disease is thoroughly brought home to the masses of the population and their economic condition so improved as to greatly restrict the opportunity for transmission of the virus. The demonstrated fact of thorough tuberculization of the race should encourage the authorities to make every effort to raise the hygienic standards by education and otherwise, as, with the resistance to the disease that the race now shows, the results of improvement in environment should be prompt and striking.

The picture of tuberculosis in the Filipino which we consider is shown in this study is that of a people long exposed to the disease, with a high mortality and morbidity. The high morbidity means a large number of carriers who, because of crowded and unhygienic living conditions, expose many contacts to massive doses. As a result, there develops a large number of fatalities in early life from primary fulminant disease, while those who survive show a high rate of relatively gross lesions. Survival requires the development of a high degree of resistance, which is shown by the preponderance of the fibrous type of lesions even in the relatively young and in the cases of recovery from periods of activity.

[8] *Saro*, an earthenware jug.

[9] *Lumbó*, a drinking vessel of cocoanut shell.

Undernutrition, intestinal parasites, malaria and bacterial infections of the intestine (typhoid and dysentery) are the predisposing causes for the recrudescences or periods of activity.

CONCLUSIONS

1. Filipinos have approximately two and a half times as much tuberculosis as Americans.

2. The natives of the Philippines, with the possible exception of the uncivilized tribes, are highly tuberculized, and the high continuing mortality and morbidity are due to poor hygiene and inadequate sanitation.

3. Morbidity and mortality in the upper age groups are particularly high as compared with both the white and colored populations of the United States.

4. The trend of tuberculosis mortality in the Philippines is now (1923) apparently upward and, while some of this may be due to better diagnosis, faults of hygiene and sanitation must bear most of the blame for preventing the rates in this people from following the downward course of the rates of other civilized countries.

BIBLIOGRAPHY

Britten, R. H., and Sydenstricker, E.: Mortality from pulmonary tuberculosis, U. S. Public Health Reports, 1922, xxxvii, 2843.

Bushnell, G. E.: Epidemiology of tuberculosis, New York, Wm. Wood & Company, 1920.

Callender, G. R., Shepard, C. A., and Kirkwood, R. C.: Tuberculosis in Filipino troops, Milit. Surg., 1925, lvi, 579.

Calmette, A.: Tubercle bacillus infection and tuberculosis in man and animals, Trans. by Soper and Smith, Williams & Wilkins, Baltimore, 1923.

Census Office, Philippine Islands, 1921, Census of the Philippine Islands, 1918, (In four volumes), Vol. ii, Population and mortality, Manila, Bureau of Printing.

City of Boston, Health Department, 1924, [Report of Framingham experiment], Monthly Bulletin, vol. 13, no. 2, February, 1924.

Gilman, P .K.: A report on the first one hundred autopsies at the Philippine Medical School, Philip. Jour. Sci., 1908, 3 (B), 211. [Discussion] 261–264.

Guyer, M. F., and Smith, E. A.: Experiments in production of typhoid agglutinins in successive generations of rabbits, Jour. Infec. Dis., 1923, xxxiii, 498.

Mendoza-Guazon, M. P.: Study of the anatomicopathologic lesions in one thousand Filipino children under five years of age, Philip. Jour. Sci., 1917, 12(B), 51.

Musgrave, W. E., and Sison, A. G.: Tuberculosis among Filipinos; a study of one thousand cases of phthisis, Ibid., 1910, 5 (B); 313.

Philippine General Hospital, 1922, Annual report of the Director of the Philippine General Hospital for the calendar year 1921; and 1923, Annual report of the Director of the Philippine General Hospital for the calendar year 1922, Manila, Bureau of Printing.

University of the Philippines: n.d., Protocols of the Department of Pathology, College of Medicine.

GOLD-SODIUM-THIO-SULPHATE

CALLED SANOCRYSIN BY MOLLGAARD

A. J. GELARIE AND F. R. GREENBAUM[1]

Gold-sodium-thio-sulphate, which Professor Møllgaard has called *sanocrysin*, is by no means a new compound. A mixture of dilute gold-chloride solution with a dilute sodium-thio-sulphate solution was employed in the middle of the nineteenth century in the Daguerre photographic process. Fordos and Gelis (1), two French chemists, investigated this mixture, and succeeded in isolating a complex gold salt which was found to be gold-sodium-thio-sulphate. Its composition was determined as $Au(S_2O_3)_2 \cdot 3Na$, that is, one molecule of auro-thio-sulphate with three molecules of sodium-thio-sulphate, or $Au_2S_2O_3 \cdot 3Na_2S_2O_3 \cdot 2H_2O$. We deal here with a complex salt which can be shown by acidification with hydrochloric or sulphuric acid, whereby no sulphur is precipitated and no sulphur dioxide is produced. This complex gold compound will not be reduced by ferrous chloride, ferrous sulphate, oxalic acid, stannous chloride, etc., to metallic gold. Hydrogen sulphide and ammonium sulphide precipitate gold sulphide. We have also noticed that gold-sodium-thio-sulphate is somewhat decomposed by the influence of light, especially when in solution. A solution should be kept in a brown or blue bottle, otherwise a yellowish powder will deposit after a certain length of time.

Using the very incomplete information of Fordos and Gelis (1) on how to make gold-sodium-thio-sulphate, we have performed a number of experiments in an attempt to prepare it. We found that, in order to get high yields, a very high concentration of sodium-thio-sulphate and gold-chloride solution is required. The addition of gold-chloride is carried out slowly under continuous, rapid, mechanical stirring. The following proportions were found to give satisfactory results:

Thirty grams of gold-chloride (light) were dissolved in 25 cc. of water (120 per cent solution of $AuCl_3$) and put into a dropping funnel, while 105 gm. of sodium-thio-sulphate were dissolved in 120 cc. of water

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(87 per cent solution of $Na_2S_2O_3$), the solution filtered and then agitated by means of a small electric stirrer. The gold-chloride was added gradually, a few drops at a time, then stirred until the yellow color disappeared, when a few more drops of gold-chloride were added. The solution assumed a milky white appearance, due to the precipitated sulphur and the colloidal sulphur, and gave a strong odor of sulphur dioxide. The addition of gold chloride lasted about half an hour and the stirring was continued for a little while. Then this milky liquid was filtered once to free it from the precipitated sulphur. After filtration, however, it still possessed a milky white appearance, due to the finely suspended colloidal sulphur. We got rid of this colloidal sulphur in the course of purification, as it is very difficult to remove it by repeated filtration.

This milky fluid was then stirred, and while being stirred received an addition of about two and a half times its volume of 95 per cent alcohol. A white precipitate was obtained after a little stirring. The addition of 95 per cent alcohol causes a precipitation of the gold-sodium-thio-sulphate, which is very readily soluble in water but insoluble in alcohol. At the same time the colloidal sulphur was also precipitated by the alcohol. This white precipitate was then dissolved in as little water as possible, the solution filtered (and in this way freed from the colloidal sulphur), and the clear filtrate reprecipitated with 95 per cent alcohol. The white precipitate, filtered off from the alcohol, can be redissolved and reprecipitated as before, to obtain a perfectly pure substance. The alcohol of the first, second and third precipitations was allowed to stand for at least 24 hours, when a considerable quantity of gold-sodium-thio-sulphate was precipitated. The gold-sodium-thio-sulphate crystallizes in colorless needles, if crystallization is allowed to proceed slowly. The method we used, however, gave a crystalline white compound. The product thus obtained was dried in air. It represents $Au_2S_2O_3 \cdot 3Na_2S_2O_3 \cdot 2H_2O$.

Thirty grams of gold-chloride gave a yield which varied in the experiments from 45 to 50 gm. of gold-sodium-thio-sulphate.

REFERENCE

(1) Fordos and Gelis: Ann. de Chim. et Phys., 1845, iii, 394.

THE EFFECT OF SANOCRYSIN ON THE TUBERCLE BACILLUS IN VITRO[1,2]

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The use of gold and its compounds in the treatment of disease dates back many centuries but, before Koch, gold therapy in tuberculosis, for our purpose at least, may be disregarded. Our actual knowledge, therefore, dates from the time that Koch demonstrated the bacteriocidal effect of potassium-auro-cyanide (KAuCN2) and other gold salts on the tubercle bacillus. From this time on, various workers have attempted to use gold in a variety of combinations.

The most important, perhaps, has been the work of Feldt and of De Witt. No attempt will be made to review the numerous publications on the subject, but only those authorities will be cited that bring out some principle bearing on the phase of the subject under discussion. Koch's work (1) demonstrated that a dilution of 1:2,000,000 prevented the growth of tubercle bacilli in bouillon cultures. He failed, however, to prevent the disease in guinea pigs. This, of course, rendered the compounds worthless for therapeutic use. An inquiry into the reason for this discrepancy led Von Behring (2) to conclude that the serum globulins interfere with the action of gold on the tubercle bacillus. He claimed that the presence of serum reduced the bacteriostatic effect from a concentration of 1:1,000,000 to 1:25,000. It was thought that for gold to act on the bacillus, it must be protected in some way from rapid ionization.

Feldt (3) took cognizance of this, and combined aurous cyanide with a detoxicated cantharidin compound, mono-cantharidyl-ethylenediamine-aurocyanide (*Aurocantan*), which had previously been found to have an affinity for tuberculous tissue. This compound was found to be bactericidal *in vitro* and to be beneficial to tuberculous rabbits, supposedly because of a dual effect, one a stimulation of connective tissue of the host, the other an oxidizing effect on the parasite and its products.

[1] From the laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois.

[2] Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

Nevertheless it was rather toxic for the animal body, due perhaps to harmful disintegration products. Following Ehrlich's example, Feldt (4) then introduced the gold into a benzole ring as the salt, p-amino-o-auro-phenol carbonic acid (*Krysolgan*).

This compound seemed to be the ideal one because it prevented growth in cultures in a dilution of 1:1,000,000, and was relatively nontoxic. With rather meagre animal experimentation, krysolgan was used in the clinics of Germany with questionable success, at first with large dosage and later with very small doses. The small doses were given for the "catalytic effect" on the tissues in destroying the tubercle bacillus. Krysolgan seems gradually to have lost favor. One outstanding fact remains, and that is that it kills tubercle bacilli *in vitro* but apparently not *in vivo*.

DeWitt (5) and her associates reopened the whole question of gold therapy, and established conclusively that the gold remedies tried were worthless, leaving the problem so far as therapeutic value is concerned just where Koch left it. Subsequently, Møllgaard (5) carried on work on gold therapy along a slightly different line. Following implicitly the principles of Ehrlich that any compound, to be of value, must actually kill the microörganisms *in vivo*, Møllgaard laid down certain requirements that must be met in order to consider an agent worthy of trial. He states, in substance, that a compound to be useful must be water-soluble, to permit of carriage to the focus of infection; it must diffuse rapidly, to penetrate the tuberculous tissue and the waxy capsule of the bacillus; the gold ions must be protected in a complex substance, and the substance must be slowly decomposed without forming free gold ions or other toxic products. Such properties he claims to have found in sodium-auro-thio-sulphate which he called *Sanocrysin*.

In the early chapters of his monograph he goes on to show that this salt conforms to all his postulates and, therefore, may lay claim to a therapeutic trial in tuberculosis. After a perusal of the work it is evident that it is not entirely complete and that certain theories have not only been unproved but seem untenable. As a complete investigation of this nature must be divided into various divisions, we have conformed to this rule and have divided the subject, part of which is a reinvestigation of Møllgaard's work, part a refinement of his work and part is entirely new. The preparation, purification and chemistry of the compound, and studies on its elimination and distribution in normal and tuberculous dogs are being done in our laboratory by McCluskey and

Eichelberger. The remainder of the work may be divided into a study *in vitro;* a study *in vivo* with emphasis on the effect of the compound on the parasite; a study *in vivo* with emphasis on the effect on the host; and from these various studies form the basis of a therapeusis, if possible, that will protect animals (and man) against tuberculosis with a dosage that may be safely tolerated by ill human beings. On this rests the future of sanocrysin therapy. It is necessary to revise the dosage used at present in patients so that a lowered mortality will be obtained; otherwise, sanocrysin is likely to go the way of Aurocantan and Krysolgan. Our first work, therefore, is a study of the effect of this drug on the tubercle bacillus *in vitro.*

EXPERIMENTAL

Bacteriostatic experiments: Varying concentrations of sanocrysin, from 1:1,000 to 1:10,000,000, were made up in a standard glycerine-agar having a pH of 7.2. The salt was added to the cooling agar in a sterile condition, so that the final volume was 10 cc., and slanted in a special rack giving a desired uniformity. After a test for contamination, a young, virulent culture of human tubercle bacilli (No. 93) was inoculated on this medium, and the tubes were plugged and lightly sealed with paraffine, capped with tinfoil and incubated at 37°C.

Table 1 shows the result of growth after 38, 53 and 67 days. There is practically complete inhibition in 1:500,000, and slight inhibition in 1:1,000,000. Below and including 1:20,000 the bacilli not only did not grow, but were definitely blackened. Microscopically, over-half of these bacilli appeared blackened, as described by Møllgaard. The blackening appeared to take place first in the granules, and in higher concentrations to spread to the bodies of the bacilli. Some bacilli show only one, two or three large blue-black granules, some of which are twice the width of the ordinary acid-fast part of the bacillus. These are no doubt similar to, or identical with Much's granules. We shall refer to them later. The same work was repeated with a bovine strain of tubercle bacilli (strain No. 198) with practically identical results. The work was repeated twice more with human strains, in dilutions between 1:20,000 and 1:50,000, to determine a sharper line of blackening. Strange to say, very little blackening was observed in these attempts, as shown by examination under the microscope. Grossly, there appeared a few crateriform colonies, having a dark gray to black depressed centre. These colonies after about 40 days died out. This shows that

this phenomenon is rather inconstant and only takes place definitely in more concentrated solutions, as was demonstrated by Møllgaard.

Glycerine-bouillon cultures, containing 5 per cent fresh horse-serum, were then inoculated with human and bovine tubercle bacilli after various concentrations of the gold salts were added. The fresh horse-serum was added to see whether there was any protective action against the salt as mentioned by others. The results are shown in figure 2. There was no growth in 1:1,000,000, and the 1:2,000,000 dilution was very slow in forming a pellicle on the surface.

TABLE 1

Showing the growth-inhibiting effect of sanocrysin on the bovine tubercle bacillus

DILUTION	TUBE A			TUBE B			TUBE C		
	38 days	53 days	67 days	38 days	53 days	67 days	38 days	53 days	67 days
1:1000	0	0	0	0	0	0	0	0	0
1:2000	0	0	0	0	0	0	0	0	0
1:5000	0	0	0	0	0	0	0	0	0
1:10000	0	0	0	0	0	0	0	0	0
1:20000	0	0	0	0	0	0	0	0	0
1:50000	∓	0	0	∓	0	0	∓	∓	0
1:100000	∓	∓	0	∓	∓	0	∓	∓	0
1:200000	∓	+	0	+	∓	0	∓	+	0
1:500000	+	+	∓	+	+	∓	+	+	∓
1:1000000	++	++	++	++	++	++	++	++	+++
1:2000000	++	+++	++++	++	++	++++	++	+++	++++
1:5000000	++	+++	++++	++	+++	++++	++	+++	++++
1:10000000	++	+++	++++	++	+++	++++	++	+++	++++
Controls	++	+++	++++	++	++	++++	++	+++	++++

In order to test further the protective action of serum colloids, a special medium was made up of three parts of fresh rabbit-serum and mixed with one part of a liquefied 6 per cent agar in a bath at 47° to 50°C. Varying concentrations of sanocrysin were added, as in the other experiments, and the tubes were rotated to mix the ingredients, slanted, inoculated, and incubated as usual. There was apparently a growth in 30 days as low as 1:50,000, but in 71 days the growth in the tubes of the lower dilutions became arrested, so that no definite conclusions could be drawn, although the blood medium does appear to enhance the growth slightly, whether by a protective action or by an improved environment we are unable to state.

Bactericidal experiments: Having confirmed the bacteriostatic effect,

the next problem was to see whether exposure of the bacilli to the drug actually killed them and, if so, in what concentrations and in what length of time. Accordingly, four dilutions, 1:2000, 1:5000, 1:10,000 and 1:20,000, of sanocrysin were made up in sterile horse-serum in corked

TABLE 2

Showing the growth of tubercle bacilli on Petroff's medium after exposure to varying concentrations of sanocrysin for varying periods of time at room temperature

A = 1:2000; B = 1:5000; C = 1:10000; D = 1:20000; E = control treated as others.

TIME		12 DAYS		20 DAYS		6 WEEKS	
2 hours.....	A	0	0	?	?	∓	+
	B	+	∓	++	++	++	++++
	C	+	+	++	++	∓	++
	D	+	+	+	++	++	++++
	E	+	+	++	++	++	++
3 hours.....	A	∓	0	++	+	++	++++
	B	+	∓	+++	++	∓	++++
	C	+	+	++	++	+	+++
	D	+	+	++	+++	+	++++
	E	∓	∓	+++	+++	+++	Lost
5 hours.....	A	+	∓	++	++	++++	++++
	B	+	+	++	++c	+	++
	C	++	++	++	++	+	++
	D	++	++	++	++	++	++
	E	+	+	++	++	++	++
13 hours.....	A	++	+	+++	++	++++	++++
	B	+++	+++	+++	+++	+++	++++
	C	+	+	+++	+++	++++	++++
	D	++	+	++	++c	++++	++++
	E	+	+	++	+++	++	++++
24 hours.....	A	++	+	++	+++	0	++++
	B	+	+	++	++c	++++	++++
	C	++	++	++	++	∓	++++
	D	+	++	++	++	++++	++++
	E	++	+	+	++	++	++++

Control—for viability: +++ ++++ +++.

centrifuge-tubes, and approximately 10 mgm. of tubercle bacilli for each cubic centimetre were added after 10, 20 and 30 minutes, 1 hour and 2 hours, respectively. Then 2 cc. were drawn off and centrifugated rapidly, and inoculated on glycerine-agar and Petroff's medium. Because

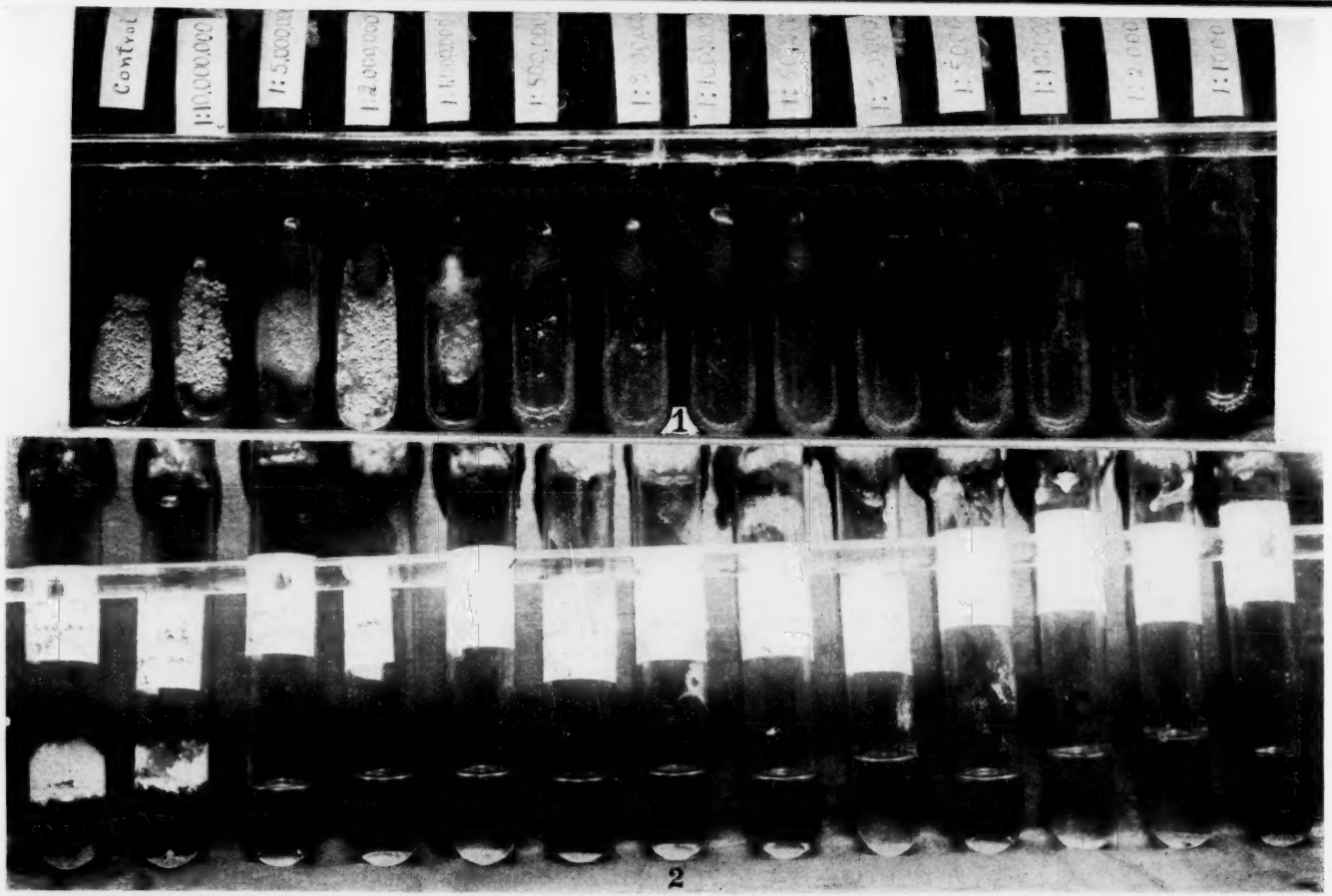

FIG. 1. GLYCERINE-AGAR CULTURES OF TUBERCLE BACILLI CONTAINING VARYING CONCENTRATIONS OF SANOCRYSIN FROM A DILUTION OF 1:10,000,000 TO 1:1000 AS SHOWN ON THE LABELS

All tubes were run in triplicate

FIG. 2. GLYCERINE-SERUM-BOUILLON CULTURES OF TUBERCLE BACILLI CONTAINING THE SAME CONCENTRATIONS OF SANOCRYSIN AS THOSE IN FIGURE 1

The control tube (resembling the one on the extreme left) was not in the photograph. A small plaque of bacilli on the 1:2,000,000 dilution (third tube from the left) does not show well.

a growth was obtained from all dilutions, as well as from controls, the experiment was considered inadequate, and was repeated, when a much smaller dosage of bacilli was used. The remainder of the experiment was identical to the previous one. The results are tabulated in table 2.

Realizing that the compound did not possess any marked bactericidal effect, the time was extended to two days, the dosage of bacilli was reduced to approximately 100,000, human serum was used instead of horse-serum, the mixture was incubated at 37°C., and at the end of the varying ntervals the bacilli were inoculated into guinea pigs. Only two concentrations were used, 1:5000 and 1:10,000, but because of a slight evaporation the concentrations were perhaps a trifle more at the end. The

TABLE 3

Showing the effect of sanocrysin on the virulence of tubercle bacilli as manifest by the type of tuberculosis in guinea pigs, produced following the exposure of the bacilli to the drug dissolved in fresh human serum

TIME OF EXPOSURE	1:5000 SANOCRYSIN			1:10000 SANOCRYSIN		
	Guinea pig number	Terminated in days	Results	Guinea pig number	Terminated in days	Results
15 minutes	J 89	51 D*	++++	J 92	85 K	++++
1 hour	J 91	45 D	++++	J 98	85 K	++++
3 hours	J 99	85 K	++	J 100	49 D	++++
5 hours	K 1	42 D	+++	K 2	Lost	—
9 hours	K 3	85 K	++	K 4	45 D	++++
24 hours	K 5	85 K	++	K 6	52 D	++++
48 hours	K 7	19 D	(?)Pn.	K 9	46 D	++++

*D = Died; K = Killed; ++++ means a generalized tuberculosis in every organ; +++ means a generalized disease in only a part of the organs; ++ means only peritoneal and glandular tubercles with a slight enlargement of the spleen.

results are given in table 3. It will be observed that, even after a 48-hour exposure in an incubator in a concentration of 1:5000 or slightly more, some bacilli still survive that are able to produce a tuberculosis in guinea pigs, even though it is only slightly more chronic than that in control animals. As this concentration is perhaps near that encountered in the body, it is evident that the theory about the "tuberculin shock" following the injection of sanocrysin must be abandoned.

It then became a question of whether the drug ever did kill the bacilli in a longer period of time. A few colonies were found to survive in the glycerine-agar cultures in a dilution of 1:20,000 for at least 40 days. It was thought expedient to test the viability for still longer periods of

time. Therefore, 0.4 cc. from the first bouillon cultures after three months' exposure was inoculated intracardially into guinea pigs.

Eighteen days later all animals were killed, and definite tuberculous lesions were observed grossly in all but one animal. The most frequent lesion was observed in the liver and hilum lymph nodes, while the spleen and lungs were definitely involved in about half the animals. In the lowest dilution (1:2000 sanocrysin) there were unmistakable lesions in the lung, liver, hilum nodes and spleen. These gross lesions were confirmed by a microscopic examination of frozen sections, but there is not yet conclusive proof that the bacilli were living or virulent. In the first place, the animals were killed too early to test the virulence of the bacilli, and, secondly, an acid-fast stain revealed only the darkly stained granular bacilli that could very well be gold-laden bacilli. The organs were reinoculated into other guinea pigs.

EFFECT OF SANOCRYSIN ON THE MORPHOLOGY AND STAINING OF THE BACILLI

From the preceding results, it is obvious that there is something that prevents the bacilli from multiplying, yet does not kill all of them outright. This is the same result obtained by DeWitt and Sherman (7) with gold chloride. The killing goes on slowly as if the bacilli were gradually being smothered. This occurs especially in concentrations that are apt to be found in the body after inoculation.

We therefore repeated the work of Møllgaard regarding the changes of morphology, and found his statement in the main correct. Various strains of tubercle bacilli were suspended in bouillon, and 1:1000 sanocrysin was added, and the tubes were incubated. All the tubes turned various shades of rose, blue and purple, but some of the cultures did not react as well as others. Thinking that the viability of the bacilli may have some influence on this reduction, a tube of bacilli was prepared and boiled before adding the gold solution. No change was observed within 24 hours, but gradually an intense blue developed that was found to be caused by a contamination, which slowly evolved presumably from spores. The next preparation was autoclaved. This one remained permanently without a blue-black color, except a very few granules in the bottom, indicating that it is necessary to have living bacilli to reduce the gold to any marked extent. The cause is perhaps a reducing enzyme. It is no doubt the same process that forms the basis of Gosio's viability test as modified by Corper (8), in which the viability of the tubercle

bacillus was determined by testing its reducing action on sodium tellurite. Another experiment was devised to throw further light on this question. A large tube of bacilli were killed by exposure under toluol 48 hours, and then a 1:1000 solution of gold salt added. The reduction proceeded as usual, but only in the bacilli at the bottom of the tube, because these bacilli did not float in the solution. In a tube prepared anaerobically the reaction took place as rapidly as it did aerobically, indicating that this process is independent of respiration.

Various other microörganisms were tested for their reducing property, and in the few at our disposal the blackening appeared only in gram-positive microörganisms. This chemical may therefore have a usefulness as a differential stain; certainly it will be useful as a test for viability. The typhoid bacillus remained free from blackening, while the staphylococcus, hay bacillus and diphtheria bacillus were blackened intensely. The diphtheria bacillus was of considerable interest. Although a concentration of 1:1000 was added, the bacillus survived and grew. At first it became blue-black; then this settled to the bottom and finally a grayish growth appeared on the surface. The gold was perhaps all used up, so that the grayish growth was free from the metal. An unstained microscopic specimen revealed numerous pairs of blue-black granules joined by a scarcely visible bridging. In fact the granules stained uniformly black.

DISCUSSION

It is almost beyond our understanding why a compound, that will inhibit the growth of the tubercle bacillus in a dilution of 1:2,000,000 and may be tolerated in the body in concentrations one hundred times that amount, should fail to produce unquestionable results. On behalf of the Danish scientists who have developed this product we can now readily understand their enthusiasm. On the other hand, it is also becoming clear why the product has not come up to expectations and has aroused so much just skepticism. As we are able to interpret the various findings, it seems that the very highly concentrated solutions (10 per cent) illustrated by Møllgaard, produce little reduction in the bacilli because of a plasmoclysis of the bacterial cells. The bacillus is dehydrated before any salt can penetrate it. The remainder of the process seems to be a quantitative affair, conforming to the mass law, with $AuSO_3$ ions and an unknown reducing substance, on the one hand, and metallic gold and other byproducts on the other. Of course, the

reaction is irreversible. In ordinary bacilli this substance is continually produced as long as the bacilli live, but in toluol-killed bacilli it reacts only within the substance present in the bodies of the bacilli at the time they were killed. In heat-killed bacilli this substance is perhaps totally or chiefly destroyed, so that practically no reduction takes place in the heat-killed bacilli.

A very significant fact is that the reduction goes on only in granulated bacilli or those bacilli that have nuclear substance. This is especially well brought out by the diphtheria bacilli and the long bacillus that was found as a contamination. Since granulation occurs in the older forms of tubercle bacilli, it seems that this reduction takes place chiefly, if not wholly, in these forms. There may be exceptions to this, but it offers a rather complete explanation of the phenomena observed. In the crateriform colonies that grew at first in the previously mentioned bacteriostatic experiments, the blackening occurred in the centre among the old bacilli. The color of bacilli roundabout gradually shaded off to the brim, where normal appearing bacilli were observed. An objection may be raised to this point because of the distance from the source of the gold salt, but in low dilutions, in which the concentration of the gold salt is not sufficient to react with the traces of reducing substances in the nongranular bacilli, no reduction is observed to take place on microscopic examination. Not until a suggestion of granulation is observed is there any reduction unless the concentration of the gold is high. Neither does there appear to be any marked amount of reduction in dead bacilli. Perhaps the salt may have an effect that tends to hasten the formation of granules, or cause bacilli to seek a resting stage, but every one so formed is eventually put out of action by the increase of gold deposited in its active living portions. Therefore only a few of the young bacilli survive and reproduce, yet rarely if ever are all the bacilli killed.

We do not mean to convey the idea that the old bacilli are exclusively attacked and the young ones exclusively exempt. It is more a matter of the presence of granules and these we have observed to occur more in the old bacilli. We have observed bacilli in patient's sputum that were entirely granular, but such specimens are rather scarce. A rather common observation is to see an involution form of bacillus completely stainless, except perhaps at one end where a rather large granule is observed. Many times the granules are seen completely free from any bacillus, and appear to be the same granules described by Much. This cannot be used in its present form as a differential stain for these granules when other microörganisms are present because the latter react as well.

It would seem that, if we are to draw any conclusions from these data, the results of treatment will depend upon the type of bacilli present. If they are of a rapidly growing nongranular type, as found in the very acute forms of disease, the results should not be as good as in the types in which the bacilli tend to granule formation. This is just the opposite to the desired result. The granular bacilli develop in the more chronic types and in those patients having a better immunity. In fact, the granulation is perhaps a vestigial structure, representing a resting or spore stage in the early phylogeny of the species, which would form when the bacillus was thrown into an unfavorable environment. These facts further demonstrate the need for caution about the idea of the compound constituting a potent sterilizing therapeutic agent. It should lead to a revision of the methods of dosage. Instead of trying to kill all the bacilli by huge doses, smaller inhibiting doses should be tried, and more reliance placed on the nonspecific stimulation of the host. The bacilli should be prevented from growing, and at the same time the patient should be kept in good condition. This would eliminate the "serum treatment" that nobody seems to be able to explain adequately—the reasons for, the action of and the benefits from it. In the first place, if the bacilli are not killed outright, there is no "tuberculin reaction" from dead bacilli. If there is no "tuberculin reaction," there is no need for such an antiserum. We do not condemn the use of an antiserum, but the necessity for it greatly weakens the cause of the method of treatment. It would seem that another explanation will have to be found for these various phenomena.

Heubner (9) attributes the reaction following gold-salt injections to an irritation of young endothelium. If this is true then the whole shock effect, at first, is a metallic poison effect, followed perhaps by a liberation of toxins from the various foci, but *not from dead bacilli*, for the bacilli do not die soon enough. Instead, there is good evidence that the salt attacks the reticulo-endothelial cells, for our recent work reveals that the material accumulates in the organs having this type of cell in abundance, such as the spleen, liver and bone-marrow.

Furthermore, the young and rapidly growing bacilli are more likely to be found outside the tubercle in the body fluids than are the old granular types. As some very recent work, which we have done, will reveal, this fact is still less favorable, because the concentration of sanocrysin becomes high in the caseous tubercles, but soon disappears from the body fluids, in measurable concentrations. The crucial part of the

whole work rests upon the ability of the drug to act on the bacilli in the body fluids, either directly or by indirect action on the cells of the host.

Let us point out here, in closing, that we have made no attempt to interpret the part played by the reaction of the sanocrysin on the host or the reaction on the bacilli in the host. That work is in progress.

SUMMARY AND CONCLUSIONS

The conclusions that we may draw from this work are: that sanocrysin inhibits the growth of the tubercle bacillus in agar cultures, slightly in 1:1,000,000 dilution and markedly in 1:500,000 dilution. In bouillon cultures it inhibits growth slightly at 1:2,000,000 and completely at 1:1,000,000. When fresh rabbit-blood agar is used as a medium there appears to be a slight growth at first even in 1:50,000 dilution, but after 40 days the growth becomes arrested. There is no noticeable bactericidal effect in concentrations as low as 1:2000 of sanocrysin for periods of two hours when about 5 billion bacilli are used, because good growth is obtained from these bacilli on Petroff's medium after the exposure. In a concentration of 1:5000, approximately 100,000 tubercle bacilli, suspended in fresh human-serum for periods as long as 48 hours, were able to produce a tuberculosis in guinea pigs only slightly more chronic and with less involvement than in control animals. Tuberculous lesions were produced in guinea pigs from the bouillon cultures after three months' exposure in concentrations as low as 1:2000 sanocrysin. At the present time, however, it cannot be positively stated whether these lesions were produced by dead or living bacilli, as it was necessary to kill the animals very early. There appears to be a reducing substance in living tubercle bacilli and some other gram-positive bacteria that is able to reduce metallic gold from sanocrysin, but this is not present in heat-killed bacilli. The gold is deposited first in the bacilli having granules, especially in the concentrations found in the body following injection of the salt. As these granules in tubercle bacilli are chiefly confined to older bacilli or bacilli in an unfavorable environment, it would seem that the detrimental effect is confined to bacilli that play more of a passive rôle in the disease. The fact that the bacilli are, at least, not quickly killed would indicate that there has perhaps been too much emphasis placed on the tuberculin-like shock following large doses of sanocrysin, as well as on its value as a sterilizing agent in therapy.

REFERENCES

(1) KOCH: Deutsche med. Wchnschr., 1890, xvi, 757.
(2) VONBEHRING, quoted by BRUCK AND GLÜCK: München. med. Wchnschr., 1913, No. 2.
(3) FELDT AND SPIESS: Deutsche med. Wchnschr., 1914, xl, 579; Berl. klin. Wchnschr., 1915, lii, 265; München. med. Wchnschr., 1914, lxi, 842; München. med. Wchnschr., 1914, lxi, 1455.
(4) FELDT: Berl. klin. Wchnschr., 1917, xlvi, 1111.
(5) DEWITT, CALDWELL AND LEAVELL: J. Infect. Dis., 1918, xxiii, 425; Trans. Nat. Tuberc. Assn., 1917, xiii, 257.
(6) MØLLGAARD, HOLGER (AND OTHERS): Chemotherapy of tuberculosis, Copenhagen, 1923.
(7) DEWITT AND SHERMAN: J. Infect. Dis., 1914, xv, 245.
(8) CORPER, H. J.: Ibid., 1915, xvi, 47.
(9) HUEBNER, W.: München. med. Wchnschr., 1913, lx, 357; Deutsche med. Wchnschr., 1913, xxxix, 690.

THE EFFECT OF INJECTIONS OF SANOCRYSIN ON NORMAL AND TUBERCULOUS DOGS[1]

Part I: On Normal Dogs

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The interest attending the introduction by Møllgaard of sodium-aurothio-sulphate (*sanocrysin*) as a treatment for tuberculosis impels further critical study of the effects of this compound upon animals before endorsing its use in the human clinic as has been done already in Denmark and England. The object of this investigation was to study the results of the intravenous injection of sanocrysin on normal and tuberculous dogs with respect to elimination, distribution in the organs, and urinary changes.

REVIEW OF LITERATURE

The British Medical Council (1) has collaborated with Møllgaard and his associates in an investigation of sanocrysin as a chemotherapeutic agent for tuberculosis, but their publications include only clinical observations. At present Møllgaard (2) is the only investigator, so far as the literature shows, who reports any effects of sanocrysin on the normal nontuberculous organism. In his report on the normal organism are included the following statements:

1. The intravenous injection of sanocrysin had no evident effect on the body temperature of the normal calf.

2. In doses of 10 mgm. per kilo body weight the intravenous sanocrysin injections in the normal calf have no visible effects on the kidney. If 20 mgm. per kilo live weight are given within short intervals, a slight albuminuria may occur. The kidneys soon accustom themselves to much higher doses.

3. After the injection of a rabbit with sodium iodide the amount of iodine excreted through the kidneys was found to be approximately 80 per cent, during a period of 5 to 8 days, in three successive experiments on the one

[1] From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois. Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

animal. This rabbit was then injected with sanocrysin, and 7 days later received an injection of sodium iodide. The recovery of iodine amounted to 91 per cent. Møllgaard concludes "That the intravenous sanocrysin injection does not diminish the excretion of the iodine. If it has any influence it seems to stimulate the excretion. As the higher animals are tolerating much higher doses of sanocrysin without getting any albuminuria, it seems justified to draw the conclusion that the intravenous sanocrysin injection in doses of 20 mgm. per kilo live weight generally has no serious influence on the excretory function of the kidneys of sound animals."[2]

4. The distribution of gold in the organs was determined after 11 injections of a 50-kilo calf, during a period of three months with a total of 17.25 gm. of sanocrysin. The animal was killed 24 hours after the last injection of 3 gm. of sanocrysin, and a chemical analysis of the organs showed that 23.6 per cent of the gold from the last injection was found in the kidney, 4.3 per cent in the liver and 1.7 per cent in the lungs.

5. A qualitative examination for gold in the urine of tuberculous patients treated with sanocrysin showed that it remained in the urine four to six days after an intravenous injection of 1 gm. of sanocrysin. Møllgaard states, "The fact that the urine contains gold 4 to 6 days after the sanocrysin injection is very interesting, because it shows that the substance remains in the body for a long time. This slow excretion is very important from a therapeutical point of view, because it augments the possibilities of the substance to diffuse into the tuberculous tissue. On the other hand, it limits the intervals between two doses to a certain minimum. If an accumulative effect in the organism shall be avoided, the intervals between two doses of 20 centigrams per kilo live weight should never be shorter than 4 days."

6. "The *dosis tolerata*, that is, the dose which has no injurious influence on the organism increases from the lower to the higher mammals." Møllgaard found the "dosis tolerata" for the following mammals to be: (1) rabbits, intravenous, 40 mgm. per kilo live weight; (2) goats, intravenous 30 mgm. per kilo live weight; and (3) calves, intravenous, 60 mgm. per kilo live weight.

EXPERIMENTAL PROCEDURE AND TECHNIQUE

For subjects, a group of 4 normal male dogs, weighing from 11 to 15 kilos, were selected and placed in metabolism cages. Throughout the

[2] The statement that sanocrysin has no unfavorable influence on the excretory power of the kidney, based upon the above data, does not appear conclusive, in view of the fact that most of the excretion of gold through the kidneys occurs during the first four to six days. Any impairment of the kidney, unless the injury be permanent, and, likewise, any changes in the excretory power of the kidney should be most prominent during the first four to six days after a sanocrysin injection. To study the changes in excretory power it would seem logical to observe the elimination of the iodine during the first six days after the sanocrysin injection; and not between the seventh and fifteenth days.

experiment these animals were maintained on a daily diet consisting of 250 gm. of raw meat, 200 cc. of whole milk and 150 gm. of bread per 10 kilos of body weight. This ration was given daily in one feeding at 3 P.M. After the dogs had been given this dietary for seven days, twenty-four-hour urine specimens were collected for six consecutive days and analyzed for chlorides, urea, creatinine and physical properties. The data obtained were regarded as average results of urinary findings on each of the normal dogs.

The dogs then received their first injection of sanocrysin.[3] The technique used in all of the injections was as follows: A 4.5 per cent solution of sanocrysin, which is practically isotonic for blood, was made by solution of the salt in sterile distilled water and injected intravenously with a calibrated Luer syringe. For each kilo of body weight 20 mgm. of sodium-auro-thio-sulphate was given for the first injection, 30 mgm. for the second, and 40 mgm. for the third.

The urine was collected every twenty-four hours, and analyzed for gold and the urinary constituents mentioned above. The results are found in tables 1, 2, 3 and 4. An injection was never given until the gold from the preceding one had disappeared from the urine.

To study the distribution of gold in the organs three of the four dogs under study were necropsied. BuM3 was killed 42 days after the third injection and 8 days after the disappearence of gold from the urine. Dogs BM4 and SM2 were each given a fourth injection of sanocrysin which consisted of 40 mgm. per kilo body weight, and necropsied two and one-half hours and twenty-four hours, respectively, after injection.

CHEMICAL METHODS

For the determination of urea in urine the urease method, with Koch's modification of the urease (4) and the Nessler solution (5), was used; for chlorides (6) and creatinine (7) in urine, the methods of Folin.

The determination of gold in urine was as follows: The 24-hour specimen of urine was measured, placed in a beaker, and evaporated over a free flame until a thick syrup of urine (approximately 50 cc.) was obtained. The concentrated urine was then transferred to a large vitresol dish (approximately 400 cc.), and heated over a free flame until completely dry. This requires about 20 to 30 minutes. To the

[3] The sodium-auro-thio-sulphate (sanocrysin) used in this experimental work was prepared by the authors (3).

TABLE 1

Percentages of gold recovered from the urine following sodium-auro-thio-sulphate injections

DAY	DOG KM1						DOG SM2						DOG BuM3						DOG BM4					
	12.99 kilos		12.33 kilos		13.14 kilos		11.60 kilos		11.89 kilos		13.43 kilos		15.83 kilos		16.23 kilos		17.57 kilos		11.07 kilos		11.49 kilos		12.70 kilos	
	First injection, 20 mgm. per kilo		Second injection, 30 mgm. per kilo		Third injection, 40 mgm. per kilo		First injection, 20 mgm. per kilo		Second injection, 30 mgm. per kilo		Third injection, 40 mgm. per kilo		First injection, 20 mgm. per kilo		Second injection, 30 mgm. per kilo		Third injection, 40 mgm. per kilo		First injection, 20 mgm. per kilo		Second injection, 30 mgm. per kilo		Third injection, 40 mgm. per kilo	
	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent	Per cent per day	Total per cent
1	21.58	21.58	17.38	17.38	19.70	19.70	25.10	25.10	19.87	19.87	25.00	25.00	26.00	26.00	20.39	20.39	23.19	23.19	19.78	19.78	27.71	27.71	17.80	17.80
2	10.61	32.19	9.52	26.90	12.40	32.10	8.55	33.65	8.82	28.69	4.5	29.5	12.58	38.58	6.46	26.85	7.0	30.19	16.31	36.09	10.51	38.22	3.10	20.90
3	8.60	40.79	0.95	27.85	7.40	39.50	5.90	39.55	4.27	32.96	1.8	31.3	6.24	44.82	4.75	31.60	9.9	40.09	5.21	41.30	3.47	41.69	3.31	24.21
4	8.50	49.29	8.73	36.58	4.30	43.80	3.60	43.15	7.93	40.89	1.6	32.9	6.04	50.86	4.42	36.02	3.9	43.99	3.33	44.66	7.30	48.99	2.74	26.95
5			6.37	42.95	3.00	46.80	3.20	46.35	4.72	45.61	2.5	35.4	1.68	52.54	4.29	40.31	1.9	45.89	5.48	50.14	3.47	52.46	3.05	30.00
6			4.10	47.05	2.81	49.61	2.20	48.55	4.45	50.06	1.4	36.8	2.37	55.91	3.30	43.61	1.1	46.99	2.54	52.68	6.17	58.63	0.70	30.70
7			1.85	48.90	1.78	51.39			2.80	52.86	1.7	38.5			1.51	45.12	1.6	48.59			1.85	60.48	1.60	32.30
8			1.94	50.84	1.17	52.56			1.20	54.06	0.8	39.3			1.25	46.37	1.7	50.29			2.25	62.73	0.70	33.00
9			1.31	52.15	1.47	54.03			0.75	54.81	0.6	39.9			1.20	47.57	1.0	51.29			1.69	64.42	1.0	34.00
10			0.85	53.00					0.84	55.65					0.83	48.40					0.74	65.16		
11			1.32	54.32	0.81	54.88			0.66	56.31	1.4	41.3			0.66	49.06	1.6	52.89			0.92	66.0	1.3	35.3
12			1.02	55.34					0.50	56.81					1.02	50.08								
13					2.09	56.93					1.2	42.5					1.5	54.39					1.5	36.8
14			2.33	57.67					0.87	57.68					2.24	52.32					1.21	67.29		
15					2.40	59.33					1.0	43.5					1.3	55.69					1.3	38.1
16			1.02	58.69					1.25	58.93					0.59	52.91					1.50	68.79		
17																								
18					2.60	61.93					1.0	44.5					1.8	57.49					1.8	39.9
19			1.33	60.02					1.20	60.13					1.76	54.67					0.69	69.48		

22					2.7	64.63					1.2	45.7					2.4	59.89					3.4	43.3
27	5.62	55.91			1.5	66.13	12.48	61.03			2.2	47.9					1.4	61.29					2.4	45.7
28	Negative		3.54	63.56			Negative		2.36	62.49			5.62	61.53	2.88	57.55			7.07	59.75	2.08	71.56		
31																							2.3	48.0
33											1.6	49.5					2.2	63.49					Negative	
34											Negative						Negative							
35					2.13	68.26																		
36					Negative																			

TABLE 2

A summary of the percentages of gold recovered from the urine after injections of sodium-auro-thio-sulphate

	DOG KM1			DOG SM2			DOG BuM3			DOG BM4		
	Total per cent recovered	Per cent recovered for first 6 days	Per cent recovered after sixth day	Total per cent recovered	Per cent recovered for first 6 days	Per cent recovered after sixth day	Total per cent recovered	Per cent recovered for first 6 days	Per cent recovered after sixth day	Total per cent recovered	Per cent recovered for first 6 days	Per cent recovered after sixth day
First injection 20 mgm. per kilo	55.91	49.29	6.62	61.03	48.55	12.48	61.53	55.91	5.62	59.75	52.68	7.07
Second injection 30 mgm. per kilo	63.56	47.05	16.51	62.49	50.06	12.43	57.55	43.61	13.94	71.56	58.63	12.93
Third injection 40 mgm. per kilo	68.26	49.61	8.65	49.50	36.80	12.70	63.49	46.99	16.50	48.00	30.70	17.30
Average	62.57	48.65	10.59	57.67	45.13	12.50	60.86	48.83	12.02	59.80	47.33	12.43

TABLE 3

Urinary findings before and after the injection of sodium-auro-thio-sulphate

PERIOD	DOG KM1								DOG SM2							
	Volume		NaCl		Urea plus ammonia		Creatinine		Volume		NaCl		Urea plus ammonia		Creatinine	
	Cubic centimetres	DN* per cent	Grams	DN per cent	Grams	DN per cent	Milligrams	DN per cent	Cubic centimetres	DN per cent	Grams	DN per cent	Grams	DN per cent	Milligrams	DN per cent
6 days before first injection	3650		10.94		38.74		2625		5840		16.68		56.44		3765	
6 days after first injection	2405	−34.1	6.17	−34.4	25.90	−33.1			4886	−16.3	13.81	−17.2	41.59	−26.3		
6 days after second injection	5733	+57.0							5680	− 2.7						
6 days after third injection	4325	+18.5	16.54	+51.1	42.14	+8.7	3131	+19.2	5435	− 6.9	16.97	+1.7	39.32	−30.3	3005	−20.2
	DOG BuM3								DOG BM4							
6 days before first injection	8580		25.86		81.70		3741		5930		13.20		46.26		3592	
6 days after first injection	6075	−29.2	18.92	−26.8	65.56	−19.7			3605	−39.2	11.05	−16.3	34.05	−26.3		
6 days after second injection	6760	−21.2							4690	−20.9						
6 days after third injection	6305	−26.5	14.86	−42.5	54.70	−33.0	3255	−12.9	4160	−30.0	9.86	−25.3	28.66	−38.0	2681	−25.6

* DN per cent = deviation from normal in per cent.

dry and somewhat charred mass concentrated nitric acid was added cautiously, and the contents ignited. If one treatment with the nitric acid does not remove all of the carbon matter a second amount is added and the ignition repeated. When all of the carbonaceous material had been removed aqua regia was added, and the contents of the dish heated to the boiling point. The solution was then diluted with water and filtered, and the filtrate made alkaline with solid sodium hydroxide. An excess of perhydrol was added, boiled gently for a few minutes, and then digested on the water bath for two hours or longer. The precipitated gold was collected on an ashless filter, washed first with water, then with hydrochloric acid (1:4), and finally with water until chloride free, ignited and weighed.

For the determination of gold in the organs the above method was used with the following modification: The organs were cut into small cubes, placed in the vitresol dish and heated over a free flame until dry and charred.

TABLE 4

Total volumes of urine expressed in cubic centimetres

DOG	FOR PRE-INJECTION PERIOD OF 6 DAYS	FOR LAST 6 DAYS OF THIRD INJECTION PERIOD	PERCENTAGE CHANGE
BuM3	8580	3985	−53.5
BM4	5930	1285	−78.3
SM2	5840	2325	−60.2
KM1	3650	4560	+24.9

EXPERIMENTAL RESULTS AND OBSERVATIONS

The experimental results are shown in the accompanying tables. Table 1 gives the percentage of gold recovered from urine per day and the total to the day following the sanocrysin injections, the weight of animal at the time of injection, the amount of sanocrysin injected per kilo body weight, and the time of the disappearance of the gold from the urine. The gold was determined in each 24-hour sample until the quantity became too small for measurement, after which two or more of the gold precipitates, as indicated by the blank spaces in the table, were united. Table 2 is a summation of table 1.

In table 3 are reported the total amounts of chlorides, urea and creatinine found in the urine, together with the total volume for 6 days preceding the first injection and for the first 6 days following each subsequent injection. Results obtained from the injection periods are

compared with the data of the pre-injection period, and tabulated as percentage deviations from the normal period. Table 5 is a report on the gold analyses of the organs.

The intravenous injection of sanocrysin into the dogs caused diarrhoea and albuminuria, and seemed to affect the vomiting centres. In nearly all instances the dogs vomited shortly after the injection. The severity

TABLE 5

Distribution of gold in organs after sodium-auro-thiosulphate injections

ORGAN	DOG BuM3, NECROPSIED 42 DAYS AFTER THIRD INJECTION AND 8 DAYS AFTER DISAPPEARANCE OF GOLD FROM URINE			DOG BM4, NECROPSIED 2½ HOURS AFTER FOURTH INJECTION OF 190 MILLIGRAMS GOLD	DOG SM2, NECROPSIED 24 HOURS AFTER FOURTH INJECTION OF 199 MILLIGRAMS GOLD
	Weight of organ, gm.	Milligrams of gold to grams of organ	Milligrams of gold found	Milligrams of gold found	Milligrams of gold found
Kidneys	114	1:33	3.4	40.3	44.2
Spleen	45	1:35	1.3	0.8	1.1
Liver	673	1:70	9.5	14.4	11.7
Pancreas	39		Trace	2.6	1.3
Bladder	21		Trace	1.0	0.8
Lungs	111	1:123	0.9	1.5	1.9
Adrenals	3.4		Trace		1.2
Heart	138	1:125	1.1		Negative
Thyroid	41		0.0		
Total			16.2	60.6	62.2
Urine between fourth injection and necropsy				28.0	50.3
Sum total				88.6	112.5

of the vomiting was in proportion to the amount of sanocrysin given. The vomitus was found to contain much bile but no gold. Albumin was present in the urine of each dog from 4 to 10 days succeeding each sanocrysin injection, and was then found to reappear at different intervals before the urine was free from gold. The amount of albumin excreted was in proportion to the weight of the gold salt injected, and varied from a trace to 1.2 gm. per litre.

PROCEDURE AND RESULTS ON CATHETERIZED DOGS

Since it was found that a rather large amount of gold was excreted through the kidneys (18 to 28 per cent) during the first twenty-four hours after the sanocrysin injection it was thought advisable to study the urinary excretion over shorter intervals of time. For this purpose three female dogs were used. Each received an intravenous sanocrysin injection of 20 mgm. per kilo of body weight, and the urine was obtained through a catheter for thirty-minute intervals over a period of four hours following the injection. The dog was then removed from the table and placed in a metabolism cage, and the urine voided was collected every two hours from the fourth to the twelfth hours, and finally

TABLE 6

Percentage of gold recovered from urine during catheterization period

MINUTES AFTER INJECTION	PER CENT OF GOLD RECOVERED		
	Dog BF1	Dog VF2	Dog SF3
30	3.33	7.24	9.33
60	1.95	6.46	7.12
90	3.52	1.81	1.46
120	6.86	1.16	0.94
150	5.19	3.62	1.12
180	2.90	1.29	2.00
210	1.80	1.55	1.00
240	0.57	2.07	3.00
Total.....	26.12	25.20	25.97

from the twelfth to the twenty-fourth hours. Each urine specimen obtained was measured and analyzed for gold. The results are found in tables 6 and 7, and graphically given in charts 1, 2 and 3.

DISCUSSION

Gold from intravenous sanocrysin injections is eliminated principally by the kidneys. An average of approximately 60 per cent of the gold was recovered in the urine for each of the dogs. This represents the minimum figure, as small losses of urine during such long periods are unavoidable.

It has been shown that in every case gold was excreted in the urine from 27 to 35 days after each sanocrysin injection. These results do not corroborate the findings of Møllgaard (2), that the gold disappears

TABLE 7

The recovery of gold from catheterized dogs

	WEIGHT	GOLD SALT INJECTED	PER CENT OF GOLD RECOVERED THROUGH												TEMPERATURE (RECTAL)		
			Catheterization		Spontaneous urination												
			4 hours		6 hours		8 hours		10 hours		12 hours		24 hours		Before injection	After 4 hours	After 24 hours
			Period	Total	Period	Total	Period	Total	Period	Total	Period	Total	Period	Total			
	kgm.	*mgm.*															
Dog BF.....	15.07	303.7 (113.5 mgm. gold)	26.12	26.12	3.25	29.37	--- ---	------→	1.05	30.42	--- ---	------→	2.64	33.06	-		
Volume of urine cc.....			170.0		75.0	252.0	70.0	322.0	120.0	442.0	0	442.0	140.0	582.0			
Dog VF 2.....	10.32	206.4 (77.2 mgm. gold)	25.20	25.20	4.52	29.72	3.23	32.95	2.58	35.53	2.58	38.11	7.10	45.21	102.5°	102.3°	102.8°
Volume of urine cc.....			519.0		196.0	715.0	140.0	755.0	40.0	895.0	130.0	1025.0	345.0	1370.0			
Dog SF3.....	13.36	266.7 (89.7 mgm. gold)	25.97	25.97	1.10	27.07	1.61	28.68	--- ---	------→	3.10	31.78	4.41	36.19	102.8°	101.9°	101.0°
Volume of urine cc.....			550.0		220.0	770.0	190.0	960.0	50.0	1010.0	40.0	1050.0	96.0	1146.0			

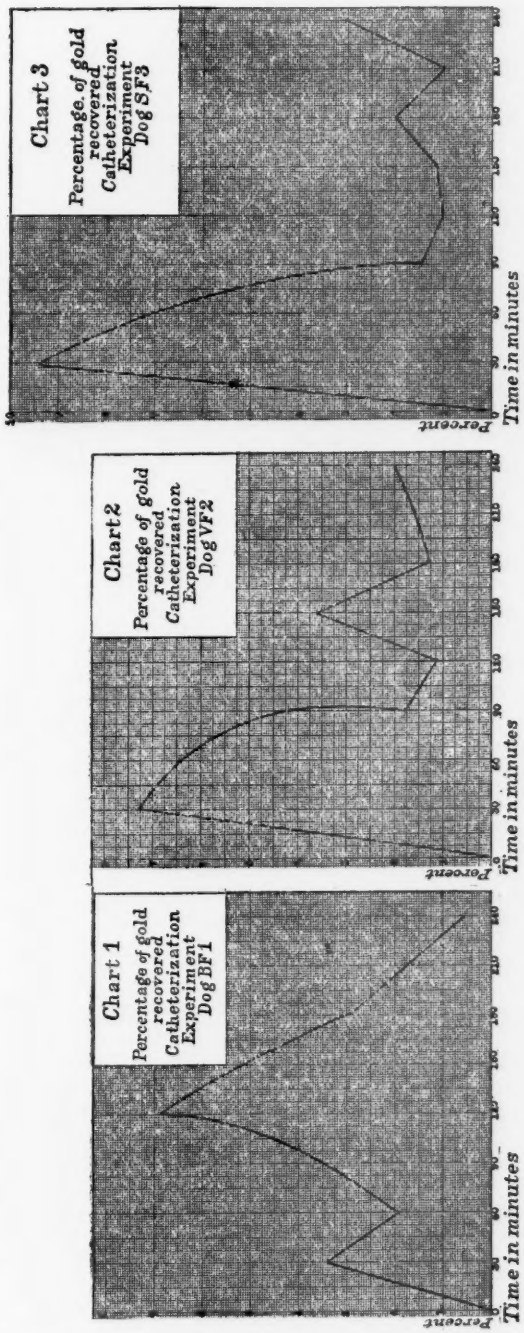
Chart 3
Percentage of gold recovered
Catheterization Experiment
Dog SF3
Percent
Time in minutes
Chart 2
Percentage of gold recovered
Catheterization Experiment
Dog VF2
Percent
Time in minutes
Chart 1
Percentage of gold recovered
Catheterization Experiment
Dog BF1
Percent
Time in minutes

in the urine in from 4 to 6 days after a sanocrysin injection of tuberculosis patients. It is true that the greater amount is excreted during the first six days, but it was found that 7 to 18 per cent has been recovered in the urine of dogs after the sixth day.

There was a small amount of gold (3 to 5 per cent) obtained from the feces during the first week after the sanocrysin injection. The animals had diarrhoea; thus some of the gold obtained was probably the result of urine contamination.

It has not been possible to isolate the gold compound from the urine, to ascertain the condition in which it is excreted. Due to the instability of the sodium-auro-thio-sulphate in the dry condition, and especially in solution, it cannot be conceived that the compound remains as such in the body for any length of time.

Most of the gold eliminated passes through the kidneys during the first six days, and therefore it would be expected that any urinary changes should be most evident at this time. For that reason a comparison of the urinary findings was made between the sixth-day period preceding the first injection with the six-day period following each injection (table 3). It will be noted that after the first injection there was, in every case, a decrease in the volume of urine from 16 to 39 per cent, in chlorides from 16 to 34 per cent, and in urea plus ammonia from 19 to 33 per cent. After the second injection there was a decrease in the volume of the urine, in three of the four dogs, of from 3 to 21 per cent. After the third injection, again in three of the four dogs, there was a decrease in all of the constituents except the chlorides in SM2, in which a slight increase (1.2 per cent) was found. Dog KM1 did not respond after the second and third injections as after the first, nor as the other dogs did. It will be noticed, though, from table 1 that this dog was the second largest in weight and therefore received more food than dogs SM2 and BM4. Furthermore, in table 3 it may be seen that the volumes, chlorides, urea and creatinine are, during the pre-injection period, considerably lower than for the dogs receiving a smaller dietary. The increases noted after the third injection may be only apparent discrepancies based on the results of the pre-injection period, which appears by comparison with the other dogs to indicate a condition not normal. If examination of the results is made on the three-day period following the third injection, even with the low pre-injection figures there is a decrease in the volume, chlorides and urea, amounting to 22, 6 and 2.5 per cent, respectively.

The question of permanent alteration to the excretory function of the kidney was considered. Toward the end of the first and second injection periods there was a tendency to return to the normal state. After the third injection of sanocrysin, which was the largest dosage, namely, 40 mgm. per kilo, an entirely different condition was present. From table 4 it may be noticed that the largest degree of oliguria yet observed was present approximately a month after the third injection. In three of the dogs there was a decrease in the volume of urine of from 53 to 78 per cent; in one dog there was an increase.

In view of the above it seems fair to conclude that the intravenous injection of sanocrysin causes oliguria and a diminution in the excretion of urea, chlorides and creatinine. Our findings do not lead to the same conclusion as reached by Møllgaard, namely, that the intravenous injection of 20 mgm. of sanocrysin per kilo body weight does not alter the excretory function of the kidney. Neither was it observed that the kidneys accustomed themselves to higher doses than 20 mgm. per kilo, as stated by Møllgaard. From the data obtained there appears to be a more permanent injury to the kidneys following the higher doses.

In an attempt to account for the recovery and distribution of all gold after the sanocrysin injections a necropsy was performed on three of the dogs. To determine the amount of deposition of gold in the organs, dog BuM3, which had received three injections of a sum total of 0.48 gm. of gold, was killed 42 days after the third and last injection of sanocrysin and eight days after the urine was free from gold. There was found a total of 16.2 mgm. of gold, or 3.38 per cent of the total gold injected, distributed in the organs as shown in table 5. This percentage of residual gold was used as an approximate factor in calculating the results from the necropsies of dogs BM4 and SM2.

From the relation between the weights of the gold found and the moist tissue (table 5) the concentration of gold in each organ was computed. The gold appeared to be most concentrated in the kidney, practically the same in the spleen, and about one-half as concentrated in the liver. The lung and the heart tissues showed very little affinity for the sanocrysin. The small amount found in the lung tissue is not an ideal condition in the practical application of sodium-auro-thio-sulphate in the treatment of pulmonary tuberculosis.

Two dogs, BM4 and SM2, were given a fourth injection of sanocrysin, containing 190 and 199 mgm. of gold, respectively. Dog BM4 was killed two and one-half hours after the injection; 28 mgm. of gold was

recovered from the urine in the bladder and 60.6 mgm. from the organs. This represents a sum total of 88.6 mgm. of gold, or a recovery equivalent to 46.6 per cent of the fourth injection, if a correction for residual gold from the previous injections is neglected. If 3.38 per cent of the injected gold from the three previous injections is assumed to be stored in the organs, the percentage recovery from the fourth injection is not 46.6 per cent, but 40 per cent, thus allowing 60 per cent of the last injection to be distributed throughout the body fluids and tissues. Since the sanocrysin disappears very rapidly from the blood it is safe to conclude that the bulk of the gold must be distributed in the body tissues.

Twenty-four hours after the fourth injection dog SM4 was killed; 62.5 mgm. of gold was found in the organs and 50 mgm. in the twenty-four hour sample of urine, making a total of 112.6 mgm. This represents a recovery equivalent of 57 per cent of the gold from the last injection, or a 49.98 per cent recovery if the correction for the residual gold is applied. Thus, it shows that, even after 24 hours, 50 per cent of the gold is distributed in the body outside of the organs analyzed. These findings prove that the greater percentage of gold is not localized in the organs but distributed throughout the tissues of the body. It is interesting to note (table 5) that the amount of gold found in the organs twenty-four hours and two and one-half hours after injection is approximately the same.

That the gold appears in the urine shortly after injection is evident from table 6, where it may be observed that the recovery of gold varied from 3 to 9 per cent during the first half-hour. In one dog, BF1, the greatest amount was excreted during the fourth and fifth half-hour periods, while in the other two this peak came in the first and second half-hour periods.

In these three dogs there was a consistent recovery of 25 to 26 per cent during the first four hours. From table 7 it will be noticed that the recovery for 24 hours was greater in every case than with the four dogs reported earlier. This may be accounted for in part by the frequent and excessive urination, caused probably through excitement and irritation by the catheter.

The rectal temperature report in table 7 shows that intravenous sanocrysin injections have no effect on the body temperature of normal dogs. This is an agreement with the findings of Møllgaard on other animals.

Microscopic Examination: Sections of the organs from the dogs

necropsied were taken for histological study. These slides were examined by Dr. Paul R. Cannon, Assistant Professor of Pathology, The University of Chicago, who submitted the following report: "The sections of the lungs, liver, spleen and pancreas of the three dogs necropsied appear to be practically normal tissues. The kidneys gave evidence of a slight interstitial nephritis. The occasional collection of round cells in the interstitial tissue indicates a subacute or chronic irritation."

The authors wish to express to Dr. Paul R. Cannon their sincere appreciation for the assistance given.

SUMMARY

1. Sodium-auro-thio-sulphate (sanocrysin) is excreted principally by the kidneys over a period of approximately 30 days.

2. The percentage of recovery of gold from the urine is practically 60 per cent.

3. Albumin, varying in amounts from a trace to 1.2 gm. per litre, was observed in all cases. The albuminuria increased with increased dosage, and lasted for a period from 4 to 10 days with subsequent reappearance.

4. The intravenous injection of the gold compound in the doses used in dogs caused diarrhoea and oliguria, and seemed to affect the vomiting centres. The vomitus contained much bile but no gold.

5. The excretion of urea, chlorides and creatinine in the urine was decreased, following an intravenous injection of sanocrysin.

6. Three to five per cent of gold was excreted through the intestine during the first week after injection.

7. The intravenous injections of sanocrysin have no effect upon body temperature.

8. The greatest amount of gold from sanocrysin localized in the organs at any time does not exceed 32 per cent of the amount injected.

9. Gold appears in the urine within 30 minutes after an intravenous injection of sanocrysin.

REFERENCES

(1) British Medical Council: Brit. M. J., 1925, vol. i.
(2) Møllgaard, H.: Chemotherapy of tuberculosis, Copenhagen, 1924.
(3) McCluskey, K. L., and Eichelberger, L.: J. Amer. Chem. Soc., in press.
(4) Koch, F. C.: Private communication.
(5) Koch and McMeekin: J. Amer. Chem. Soc., 1924, xlvi, 2066.
(6) Folin, O.: Laboratory manual of biological chemistry, New York and London, 2nd Ed.
(7) Folin, O.: J. Biol. Chem., 1914, xvii, 469.

THE DIAGNOSIS AND MANAGEMENT OF LARGE PLEURAL EFFUSIONS COMPLICATING PULMONARY TUBERCULOSIS

HERMAN HENNELL[1]

The difficulties of diagnosis and management of localized or encapsulated effusions have been pointed out and admirably discussed in recent literature on the subject. At the same time, it is realized by only a few that the diagnosis of large and relatively free effusions complicating pulmonary tuberculosis often presents great obstacles even to the expert. A careful consideration of the subject will readily convince one that the accepted diagnostic criteria for such conditions are often disappointing. The frequency with which such effusions are not recognized, and even when recognized are not infrequently mismanaged, prompts the report of some typical cases in the hope of calling attention to the points which may be helpful in their proper diagnosis and rational management.

The physical signs presented by large pleural effusions are usually clear-cut and easily elicited, even by the tyro. Inspection may reveal a slight fulness of the affected side, a smoothing out of its interspaces and diminution of its respiratory movements. In addition, the displacement of the cardiac impulse to the unaffected side may be readily noted.

On palpation, the spasm of the intercostal muscles over the fluid may frequently be felt, and diminished tactile fremitus is usually elicited.

However, it is the results of percussion which give us our first suggestive evidence of the presence of an effusion. A small amount of fluid may show itself as an area of dulness. In moderate effusion, three zones of resonance may be mapped out, namely, flatness directly over the fluid; above that, a zone of mingled dulness and tympany; and normal resonance at the summit. But with large effusions marked dulness or even flatness may be observed throughout the involved hemithorax, with the exception of a small area above the clavicle or over the pul-

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monary bronchi in front, where a high-pitched tympanitic note may be heard, provided the bronchi remain open, as they almost always do.

The auscultatory signs are even more definite. In most cases voice and breath-sounds are absent or very feeble over the effusion; while over the compressed lung above the fluid the breathing may be bronchial or bronchovesicular, according to the degree of compression. On the sound side the breathing is usually exaggerated; and the dislocation of the cardiac apex to right or left, as the case may be, is often more readily discovered by auscultation than by either inspection or palpation.

What is less well known, and still less remembered by those who know it, is the fact that in a large number of cases the breathing and voice-sounds over the fluid may be of the bronchial type, sometimes feeble, sometimes very intense, and that râles and friction sounds are not infrequently heard over it. For fluid transmits sound well, and whatever respiratory sounds are produced may be heard over it unless they are interfered with by inflammatory thickening of the costal pleura.

In the presence of a tuberculous process on the affected side, the physical signs elicited over the fluid are, for reasons to be discussed later, very much more complex; and the diagnostic criteria of marked dulness to flatness, absent or feeble breath-sounds and absence of râles over the fluid, as well as the displacement of mediastinal structures to the unaffected side, do not hold in most cases. A correct diagnosis in such instances will tax the ingenuity of the keenest clinician, and is only too frequently not made even after careful study, as the report of the following cases will show.

REPORT OF TYPICAL CASES

Case 1: M. P., age 40, presented himself with a history of pulmonary tuberculosis of eleven years' standing, and of more or less acute general and pulmonary symptoms of nine days' duration. A day later his temperature rapidly rose to 105.4°, and dyspnoea, cyanosis, and marked prostration were the other main symptoms.

Physical examination of the lungs at this time revealed what was interpreted to be an acute pneumonic process, probably caseous in nature, in the left lower lobe, superimposed upon an extensive bilateral pulmonary tuberculosis. The signs in the left lung, the one of particular interest in this discussion, were as follows: marked dulness to flatness throughout, especially over the basal lobe; harsh bronchocavernous breathing over the upper lobe and similar but less harsh breathing over the base; and coarse moist râles over the entire hemithorax and a number of coarse leathery creaks over the

lower lobe posteriorly. The heart appeared to be slightly pulled to the left, as judged by percussion and auscultation.

In the evening of the same day the patient showed the classical signs of cardiac failure with pulmonary oedema. He was pulseless, and the cardiac sounds could hardly be heard because of the numerous bubbling râles throughout both lungs; his extremities were cold and cyanosed, and he was sinking rapidly into semicoma. Treatment with adrenalin and atropine was instituted at once, and his response to this stimulation was most striking. In about an hour his pulse could be felt again at the wrist, and remained of fair quality. The dyspnoea and cyanosis diminished to a very marked degree, and the patient himself remarked how much better and stronger he felt.

However, after this spectacular improvement and a temporary rally the following day, the patient's condition again began to decline, with marked dyspnoea and prostration as the predominating symptoms. Cardiac failure with pulmonary oedema supervened and did not yield to treatment. The patient died a day later with signs and symptoms of cardiorespiratory failure, which were interpreted at the time as apparently secondary to the acute pneumonic process in the left lower lobe superimposed upon on extensive bilateral pulmonary tuberculosis.

The roentgenogram made a day before death showed a dense homogeneous shadow throughout the left hemithorax, the density shading off toward the periphery. However, the phrenic shadow and sinus appeared clear, and the mediastinal structures were not appreciably displaced to the right. This led to the interpretation of the homogeneous density throughout the left hemithorax as evidence of consolidation and thickened pleura rather than of an effusion.

There were, however, two features in the clinical record of this case which disturbed my complacency with the above diagnosis as they were strongly suggestive of some septic process: (1) the markedly septic temperature with 97.6° and 105.4° as the lowest and highest levels; and (2) the marked leucocytosis, namely, 21,400, with 89 per cent polymorphonuclears, and 35,800 with 92 per cent polymorphonuclears a day later. For this reason a lung puncture was done immediately postmortem, in order to possibly determine whether the pneumonic process in the left base was tuberculous or septic in nature; when, to my dismay, puncture after puncture at various levels from base to apex revealed abundant and somewhat turbid fluid.

Comment: In brief, here was a case, in which a large effusion complicating pulmonary tuberculosis, was overlooked because the physical signs and even the roentgen-ray findings were rather typical of a pneumonic process in the base superimposed upon an old pleuropulmonary tuberculous process, and not at all characteristic of a large pleural

effusion, as judged by our older diagnostic criteria for such conditions. Cardiac failure with pulmonary oedema, supposedly of toxic origin, appeared once and cleared up readily under vigorous treatment, only to recur two days later and prove fatal.

That such an effusion might have been suspected from some of the clinical features of this case became obvious only after its presence was demonstrated by pleural puncture. The temperature curve, the marked leucocytosis with high polymorphonuclear count, and particularly the spectacular cardiac response to stimulation during the first attack of circulatory failure could be easily explained on the basis of such a diagnosis. The last point, especially, was suggestive of a mechanical factor as the cause of the cardiac embarrassment, rather than the toxic factor which was believed to operate. Yet, while all this was readily appreciated in retrospect, I question whether a diagnosis of a large pleural effusion in this case could have been made without resorting to an exploratory puncture, a procedure which is entirely justified in doubtful cases of this kind.

Case 2: R. R., age 25, steam-fitter, gave a history of an acute illness six months before, with pain in the right side, cough, dyspnoea and fever of 105°, as the main symptoms at that time. The condition was originally diagnosed as pneumonia, and the patient recovered considerably after six weeks' stay in the hospital, except for a slight persistent cough. However, about six weeks later his cough became aggravated, and the rapidly increasing dyspnoea forced him again to enter a hospital for treatment. Soon after admission to the hospital his right pleural cavity was aspirated, and about 4000 cc. of moderately turbid fluid were withdrawn, which, on examination, revealed numerous tubercle bacilli. After about two months of convalescence he was again complaining of a slight cough, moderate dyspnoea on exertion, and occasional pains in the right side of the chest.

Physical examination of the chest at this time showed a probable slight involvement of the left upper lobe and the following physical signs on the right side: On inspection there was apparent shrinkage, and diminished respiratory mobility of the entire right hemithorax. Palpation revealed the trachea pulled considerably to the right, the interspaces somewhat narrowed, especially in the upper portion of the thorax, and tactile fremitus somewhat diminished. Percussion elicited marked dulness over the upper third, and flatness over the lower two-thirds of the hemithorax. On auscultation, bronchial breathing, considerably increased whispered voice and numerous moist râles were heard from the apex to the level of the third rib; while, below, the intensity of the breath-sounds decreased rapidly, and numerous fine

râles and squeaks could be heard over that portion of the lung. The apical cardiac sounds were audible in approximately their normal position, and there appeared no corresponding displacement of the heart toward the affected side, as was observed in the case of the trachea.

The roentgen-ray examination at this time revealed a marked and fairly homogeneous density throughout the right lung field, with the shadow somewhat less dense above the clavicle. The trachea was definitely pulled to the right. On the left side there was evidence of increased hilum and peribronchial fibrosis. The cardiac shadow appeared quite prominent and somewhat pear-shaped, but apparently not displaced either to right or left.

Obviously, the physical and roentgen-ray findings in this case favored the view that an extensive pleuropulmonary process existed on the right side, with probable fibrosis and cavitation in the upper lobe and an effusion in the process of absorption in the basal field. However, as an exploratory puncture showed the presence of moderately turbid fluid, aspiration was decided upon, and 800 cc. of the fluid were withdrawn, some of which was replaced by air for reasons to be discussed later.

A roentgenogram, made following the aspiration, revealed that the shrinkage of the right hemithorax and the displacement of the mediastinal structures toward the affected side, which were the deciding factors in favoring the diagnosis of a pleuropulmonary process rather than an extensive effusion, were not erroneous. That this consideration should not deter one from making the latter diagnosis became obvious when the roentgenogram showed the marked fibrotic changes in the underlying lung which, it is fair to assume, had to a large extent antedated the occurrence of the effusion, and had so fixed the mediastinal structures in their displaced positions that the existing intrapleural pressure could not appreciably affect it.

Comment: The importance of a correct and early diagnosis of such conditions is not merely of academic interest; indeed, it has a direct bearing on their proper management. As I shall point out later, in the discussion, it is my conviction that the only rational treatment of these effusions consists in repeated aspirations in order to avoid the destruction of the function of the underlying lung, through complete compression fibrosis, and even calcification which may result under such circumstances.

The danger of such a result will be readily appreciated, if it is remembered that not infrequently a more or less extensive tuberculous process, dormant or slightly active, is present in the other lung. And when the entire function of respiration is thrown on this already impaired organ, earlier or later, the tuberculous process therein may become active.

Under these circumstances, with one lung entirely crippled and functionless, the tuberculous process in the other lung may become rapidly progressive, and lead to a fatal issue in a very short time, as will be shown by the following case.

Case 3: E. F., age 22, bookkeeper, presented himself with a history of acute onset of marked pulmonary symptoms about ten months before. After three weeks of severe illness he was taken to a hospital. He showed, at that time, a very extensive pneumonic phthisis on the left side, with practically no involvement of the right lung. Because of the acuteness of the process and its rapid progress, artificial pneumothorax was induced on the left side, and a good collapse of the affected lung was achieved after a few refills. However, as an effusion soon developed, further inflations were deemed inadvisable; and, as the patient showed signs of consistent improvement, the fluid was left undisturbed. When the patient first came under my observation his only complaints were slight productive cough and considerable dyspnoea on slight exertion. Physical and roentgen-ray examinations at this time revealed a very extensive left pleural effusion, with almost complete dextrocardia as a result of it.

Because of the increasing dyspnoea, which was evidently due to the cardiorespiratory embarrassment resulting from the extensive effusion on the left side, and in accordance with the accepted practice in dealing with such conditions (1), the fluid was aspirated in three stages, about 3500 cc. being removed altogether.

A roentgenogram, taken following the aspiration of the fluid, revealed a markedly compressed and apparently fibrosed, as well as calcified, left lung. That this organ was apparently functionless was obvious from its failure to change at all with inspiration and expiration, as observed roentgenoscopically. The right lung showed probable infiltration along the increased radiation from the hilum region into the upper lobe.

As the fluid at first showed a tendency to reaccumulate, frequent aspirations were resorted to, in order to avoid, as much as possible, embarrassment of the cardiovascular system and the other lung by undue displacement of the mediastinal structures. And, in order to obviate too marked changes in the intrathoracic equilibrium, during and immediately following aspiration of the large amounts of fluid, as well as to prevent subsequent chest deformity, some of it was replaced by air.

Under this treatment the patient did very well for seven months following, when he suddenly took sick with fever of 103°–104°, marked prostration, and increased cough. About two weeks later, an extensive tuberculous process on the right side was discovered by physical and roentgen-ray examinations. From this time on his condition declined rapidly, and he died six weeks later,

at which time physical and roentgen-ray examinations revealed extensive tuberculosis of the entire right lung in addition to the older findings on the left side.

Comment: It is fair to speculate that had the patient's pneumothorax and the subsequent effusion been so managed as to prevent the complete annihilation of the function of that left lung, the latent tuberculous process in the untreated lung might have been kept in check for a much longer time, since it would not have been forced to perform the entire function of respiration unaided.

DISCUSSION

From the foregoing it is seen that the physical and roentgen-ray findings in the cases presented were sufficiently "irregular," differed just enough from the classical picture of large effusions, to make a correct diagnosis either difficult or impossible. It will, therefore, be of interest to review these data critically in an effort to rationalize them.

Physical diagnosis of the lungs is, to a very large degree, based on the general laws of acoustics. Without going into too much detail in the discussion of the principles of acoustics as applied to pulmonary phenomena, a few of these principles must be thoroughly appreciated and understood, in order to understand the physical signs produced by large effusions complicating pulmonary tuberculosis.

The interpretation of the physical signs elicited from healthy or diseased lung tissues is based on the circumstance that physical alterations of these tissues cause corresponding changes in the sound vibrations which they are able to create or transmit. While the question of the mode of production of the breath-sounds as heard over the chest is still much debated, recent evidence (2) seems to prove conclusively that the origin of the respiratory sounds, inspiratory and expiratory alike, is in the larynx. According to this conception, the sound produced at the place of origin, the larynx, is a mere noise, some element of which is capable of exciting the sympathetic resonance of the thorax. And it is this principle of the selective action of resonators which plays such an important part in determining the characteristics of the sound within the respiratory apparatus. For instance, the specific character of vesicular breathing is due to the resonator action of that part of the normal thoracic cavity, and its contents, over which such breathing is heard. If pathological changes occur in that part of the thorax or

its contents, its resonator and transmitter qualities are accordingly altered, and bronchial, bronchovesicular or cavernous breathing may replace the normal vesicular murmur. That the mode of production of râles can be similarly explained is claimed by a number of observers, and with good reason.

Bearing these fundamental principles in mind, it may be easily comprehended why, in the presence of a tuberculous process the physical signs elicited over a superimposed effusion are so complex; for the greatly altered lung structures, as well as the number and variety of adhesions usually present, act as peculiar resonators, modifiers and transmitters of the original tracheal sound. And as fluid transmits sound well, bronchial or cavernous breathing, voice and whisper, as well as râles of all descriptions, may be heard over it, provided the physical conditions in the thorax are favorable for their production and proper transmission.

The failure of the mediastinal structures to be shifted to the unaffected side, which occurs in a number of such cases, is more readily explained. For it is to be remembered that, in the type of cases under discussion, the effusion is frequently superimposed upon an old tuberculous process where extensive fibrosis has either previously pulled over the mediastinal structures to a marked degree, or at least has so fixed them by adhesions that no amount of effusion is capable of appreciably affecting their displacement. It may therefore happen that, instead of a shifting of the mediastinal structures toward the unaffected side, physical examination may reveal them either in their normal position, or even drawn to the side of the effusion.

The roentgen-ray findings are, as a rule, of great assistance in the diagnosis of such conditions; but not infrequently a differential diagnosis between a large effusion and a markedly thickened pleura presents real difficulties. This is particularly true when, because of the marked fibrotic changes in the lung underlying the effusion, the mediastinal structures are displaced toward the affected side. However, the marked density and homogeneity of the shadow, which usually fades somewhat toward the periphery and apex in proportion as the antero-posterior diameter of the chest diminishes in that direction, is strongly suggestive of fluid. Moreover, because of the gradual diminution of the depth of the fluid column in and about the region of the sinus, the phrenic shadow may be faintly but definitely outlined as a result of shadow contrast. Finally, the absence of suggestive evidence of cavitation, as indicated

at which time physical and roentgen-ray examinations revealed extensive tuberculosis of the entire right lung in addition to the older findings on the left side.

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The roentgen-ray findings are, as a rule, of great assistance in the diagnosis of such conditions; but not infrequently a differential diagnosis between a large effusion and a markedly thickened pleura presents real difficulties. This is particularly true when, because of the marked fibrotic changes in the lung underlying the effusion, the mediastinal structures are displaced toward the affected side. However, the marked density and homogeneity of the shadow, which usually fades somewhat toward the periphery and apex in proportion as the antero-posterior diameter of the chest diminishes in that direction, is strongly suggestive of fluid. Moreover, because of the gradual diminution of the depth of the fluid column in and about the region of the sinus, the phrenic shadow may be faintly but definitely outlined as a result of shadow contrast. Finally, the absence of suggestive evidence of cavitation, as indicated

by circumscribed areas of increased transmission of the ray, is another helpful point in favor of an effusion.

However, a correct diagnosis in such cases will be established only by a careful study of the clinical history, an intelligent interpretation of the physical signs elicited in the light of our modern conception of the principles of acoustics as applied to pulmonary phenomena, and by painstaking roentgen-ray studies. And in some puzzling cases an exploratory puncture may be the only means of reaching a final decision.

As has already been pointed out, a correct and timely diagnosis of these effusions is not merely of theoretical interest, but is of supreme importance in the proper management of such cases. The time is past when large effusions, whether complicating pneumothorax or not, are left to their own fate. Such a policy has proved disastrous in too many instances, and it has been abandoned by the best workers. The indication for treatment of such conditions is aspiration, to be repeated as often as may be necessary. The rationale of this procedure is too obvious to require an elaborate argument. In some instances, the cardiorespiratory embarrassment is so acute that, unless early relief by aspiration is instituted, a fatal issue may result. Case 1 of our series may serve as an illustration of this type.

However, in most instances the immediate cardiorespiratory embarrassment is less acute, and the danger of neglecting aspiration of these effusions is proportionately greater. That there is real danger in allowing large effusions to press upon the underlying lung for long periods of time has been illustrated by two of the cases. It will be recalled that in both instances quoted, following the aspiration of long-standing effusions, there was found a lung so thoroughly collapsed, fibrosed and in one instance calcified, that it was practically rendered functionless. From the previous clinical histories of these cases it was fair to infer that the extent of disease of the lung in question was probably not extensive enough to warrant a procedure which eventually led to a complete and permanent loss of its function.

The danger of complete compression of a lung with subsequent fibrosis and possible calcification has been pointed out elsewhere (3), and is now thoroughly appreciated by the most enlightened workers in the field of artificial pneumothorax, so that moderate collapse with partial pneumothorax is practised in all instances in which the necessary conditions are present. That danger is due to the fact that latent disease in the other lung may be stirred into activity when it is com-

pelled to perform the entire function of respiration unaided. And should the process there become active and progressive, the prognosis would be very grave indeed, as was illustrated by case 3 of the series.

On the other hand, early and frequent aspirations will prevent an undue collapse and subsequent fibrosis of the underlying lung; and, following the cessation of the accumulation of fluid, it may be allowed to expand or it may be treated with "partial pneumothorax," as the condition may demand. It is for this last-mentioned reason, and some others presently to be stated, that in the treatment of these conditions it is advisable to replace some of the aspirated fluid by air. This procedure has proved of great value, inasmuch as the air bubble, by converting the effusion into a hydropneumothorax, clearly defines the limits and volume of the effusion, and makes subsequent fluoroscopic studies as well as aspirations relatively easy. Moreover, marked disturbance of the intrathoracic equilibrium, with consequent cardiorespiratory embarrassment, which may be occasioned by the aspiration of large amounts of fluid, is obviated, if part of the fluid is replaced by air, a consideration which has been discussed in detail elsewhere (4).

SUMMARY AND CONCLUSIONS

1. Large effusions complicating pulmonary tuberculosis are frequently not recognized because the diagnostic criteria of (1) marked dulness to flatness, (2) feeble or absent breath-sounds, (3) absence of râles over the fluid, and (4) shifting of the mediastinal structures to the unaffected side, do not hold in the majority of such cases.

2. It is to be remembered that fluid transmits sound well; and bronchial or cavernous breathing voice and whisper, as well as râles of every description, may be heard over it, provided the physical conditions in the thorax are favorable for their production and proper transmission.

3. In the presence of a tuberculous process on the affected side, the physical signs elicited over the fluid are very complex, because of the greatly altered lung structures, as well as the number and variety of adhesions usually present, which act as resonators, modifiers and transmitters of the tracheal sounds.

4. The displacement of the mediastinal structures to the unaffected side is not infrequently absent, and these structures may even be pulled the other way in the presence of an extensive fibrotic process in the lung on the affected side.

5. While the roentgen-ray findings are usually of great assistance in such conditions, they are occasionally misleading, especially when the mediastinal structures are pulled to the affected side by marked fibrotic changes in the lung underlying the effusion, a diagnosis of thickened pleura being usually made under such circumstances.

6. A correct diagnosis in such cases will be established by careful study of their clinical history, an intelligent interpretation of the physical signs elicited in the light of our modern conception of the principles of acoustics as applied to pulmonary phenomena, by painstaking roentgen-ray studies, and in doubtful cases by exploratory puncture.

7. The indication for the treatment of these effusions is aspiration, repeated as often as necessary, in order to relieve pulmonary and cardiovascular embarrassment in some cases, and prevent complete compression and fibrosis of the underlying lung in others, in which there may be good reason to fear such a result, such as the presence of disease on the unaffected side or comparatively little disease on the affected side.

8. The replacement of some of the fluid by air is a valuable procedure, inasmuch as it clearly defines the amount and limits of the effusion and makes subsequent aspiration much easier. Besides, aspiration of large amounts of fluid, if part is replaced by air, disturbs the intrathoracic equilibrium little, and possible cardiorespiratory embarrassment is avoided.

9. By converting the effusion into a hydropneumothorax, the pneumothorax may be continued following the cessation of the accumulation of fluid, or the lung may be allowed to expand completely, as the circumstances then may warrant.

REFERENCES

(1) Stivelman, B., and Rosenblatt, J.: J. Am. M. Ass., 1921, lxxvii, 12.
(2) Bushnell, G. E.: Ibid., 1921, lxxvii, 2104.
(3) Hennell, H., and Stivelman, B.: Amer. Rev. Tuberc., 1923, vii, 291.
(4) Stivelman, B., Hennell, H., and Golembe, H.: J. Am. M. Ass., 1922, lxxviii, 1450.

THE QUESTION OF THE TRANSMUTATION OF TUBERCLE BACILLI AND PARATUBERCLE BACILLI[1]

A. CALMETTE

A large number of varieties of bacilli morphologically resembling the tubercle bacilli are known to exist. All these bacilli possess the property of resisting decolorization by acids and alkalis after they have been stained with Ziehl's fuchsin.

These bacilli, so called acid-fast, or *paratuberculous*, are commonly found in widely different situations. Some among them are pathogenic for certain animals or for man. Such include Johne's bacillus of hypertrophic enteritis in cattle, Hansen's bacillus of human leprosy, Stephansky's bacillus of rat leprosy, the bacillus isolated from pseudotuberculous tumors in the carp by Dubard, Bataillon and Terre in 1897, the bacillus found in the adder by W. K. Sibley in 1889, that of the tortoise discovered by Friedmann, and that described by Marchoux in 1921 under the name of *Mycobacterium pulviforme* which he discovered in the organs of a pseudoleprous patient from Hayti.

For the most part such bacilli consist of the saprophytes which, in nature, take part in the breaking down of carbohydrates and perhaps also of fats.

Among these several have been the object of special study. For example, the timothy-grass bacillus of Moeller (1898), the manure or *Mist-bacillus*, also of Moeller, the various bacilli isolated from milk and butter (the bacillus of Petri, of Rabinowitsch, of Korn, of Coggi, of Tobler, of Markl, of J. Binot, of Beck, etc.), from sewage (Houston), from human and animal excreta (Severin, Capaldi, J. Ferran, Cramer, de Giacomi), from the excretions of cutaneous and mucous surfaces (Gottstein, Alvarez and Tavel, Matterstock, Bitter, Laabe, Moeller), from taps, rubber tubes, metallic instruments, especially trumpets (Jacoby and Kayer, Schmitz, Bruno Lange), etc.

[1] This paper was presented at the Fourth Conference of the International Union against Tuberculosis, held at Lausanne, Switzerland, August 5–7, 1924, as a report on the biological question, "Do there exist in nature or can there be artificially produced saprophytic varieties of Koch's bacillus which can become virulent tubercle bacilli?" It has also been published in the volume of the proceedings of the Congress, pp. 51–73.

It is natural to inquire whether these microbes, possessing such close resemblance to Koch's bacillus, are not the result of the transformation of the authentic bacillus of tuberculosis under the influence of environment, or whether they arise as the adaptation of true tubercle bacilli to a saprophytic existence.

On the other hand it might be supposed that the tubercle bacillus represents the progressive adaptation of a single acid-fast saprophyte to a parasitic life in the body of a warm-blooded animal.

These two hypotheses have been discussed for a quarter of a century without any worker furnishing convincing proofs in favor of either. It appears, therefore, very desirable that we see whether it is possible that, either by evolutionary change or by sudden transformation, a "paratubercle" bacillus can acquire the tuberculosis-producing properties which are characteristic of the tubercle bacillus. This question is of the greatest importance for establishing prophylactic measures against tuberculosis.

1. MORPHOLOGICAL AND PHYSIOLOGICAL FEATURES OF THE PARATUBERCLE BACILLI

On microscopic examination, after staining by Ziehl's method, most of the saprophytic paratubercle bacilli present the same appearance as the tubercle bacilli. A certain number, particularly those found in the soil, in sewage and in animal dejecta, are, however, fatter and shorter, often ovoid in shape, sometimes branching, arranged in stellate groups, or club-shaped.

Nearly all are easily cultivated on the usual glycerine or glucose media. They grow in a mass at the bottom of the vessel, or in films which are generally thick, wrinkled and more or less colored either a lemon-yellow or a brick-red. A culture can be obtained in a few days, in some cases even in a few hours, at temperatures of from 25° to 30°C.; they will also grow at 38°, and even in the neighborhood of 65°, although very slowly.

They do not liquefy gelatine or coagulate milk. In liquid gelatine media they give rise to a slight odor of trimethylamine or of ammonia, which is very different from the jasmine-like smell produced by tubercle bacilli.

Reagents which dissolve fat and wax (chloroform, toluol, acetone) destroy the acid-fast properties of the paratubercle bacilli more quickly than in the case of Koch's bacillus, and the former are more easily de-

colorized than the latter by acids, alkalis, sodium sulphite, and even by simple immersion in boiling water for two to three minutes.

If the tubercle bacillus and the timothy-grass bacillus, cultivated on the same medium (glycerine-broth) and carefully freed from all water-soluble and alcohol-soluble material, are hydrolyzed by means of sulphuric acid, as has been done by A. Berthelot at the Pasteur Institute, it will be found that the amounts of reducing sugar obtained from 100 gm. of dry bacteria vary markedly according to the species studied. It is found that

Bovine tubercle bacilli give 6.8 gm. of reducing sugars per 100 gm. of dry bacteria.

Human tubercle bacilli give 5.87 gm. of reducing sugars per 100 gm. of dry bacteria.

Avian (turkey and fowl) tubercle bacilli give 1.69 gm. of reducing sugars per 100 gm. of dry bacteria.

Timothy-grass bacilli give 0.75 gm. of reducing sugars per 100 gm. of dry bacteria.

Certain saprophytic paratubercle bacilli will produce lesions if they be inoculated into, or if they be ingested in large doses by warm-blooded animals. Such lesions present a very definite appearance. They consist of actual masses of bacilli and leucocytes coated with fibrin, which form pseudotubercles, with or without giant cells, but which rapidly disappear by reabsorption or by intracellular digestion, and which are never re-inoculable in series into other animals of the same species. Sometimes they remain for a long time unchanged at the seat of inoculation as well as in the lymph nodes, spleen, liver or kidneys of the inoculated animal. Bruno Lange (1) has been able to recover bacilli which have not lost their vitality after 32 weeks. Successive passage through rabbits or guinea pigs does not appear to increase their virulence. The only effect produced is to render them susceptible to more rapid cultivation at a temperature of 38°.

The paratubercle bacilli are feebly agglutinated by animal serum prepared with homologous bacilli. They are not agglutinated at all, or only with difficulty, by serum from a tuberculous animal.

On the other hand, they can be used as antigens for detecting tuberculous antibodies in the complement fixation reaction of Bordet-Gengou. Extracts with methyl alcohol treated with acetone are always, as Boquet and Nègre have shown, less active than those from tubercle bacilli.

human, bovine or avian. It must be remembered that alcoholic extracts of *B. subtilis*, of *Corynebacterium diphtheriae* and even of fungi, such as the *cryptococcus* of Rivolta, are equally capable of fixing complement in the presence of serum from a tuberculous patient.

In culture media the paratubercle bacilli produce substances analogous to tuberculin. Glycerine extracts can be prepared which, when injected subcutaneously or intracutaneously in large doses, give rise to a febrile reaction or to local reactions similar to those obtained with tuberculin. This is better seen in human tuberculous subjects than in the case of animals.

The bacilli themselves are, however, practically nontoxic for the tuberculous animal, even on intracerebral injection (A. Borrel, Pinoy and Burnet). They are, nevertheless, capable of sensitizing the body. They can be used for carrying out intracutaneous reactions in guinea pigs infected with Koch's bacillus. They give rise to positive reactions, provided larger doses are employed than in the case of the human or bovine tubercle bacillus.

Repeated injections of the paratubercle bacilli into healthy animals will sensitize them against the same microörganisms and will give rise, as in the case of the tubercle bacillus, to Koch's phenomenon, with the formation of small cold abscesses, which open freely on to the surface and which heal rapidly. Enlargement of the lymph nodes in the neighborhood of the site of the first inoculation is not seen.

2. ATTEMPTS TO TRANSFORM PARATUBERCLE BACILLI INTO TUBERCULOGENIC BACILLI

Up to the present time the only workers who claim to have succeeded in inducing tuberculosis in mammals with paratubercle bacilli are, on the one hand, Dubard, Bataillon and Terre with their piscine bacillus and, on the other hand and more recently, W. Kolle, H. Schlossberger and W. Pfannenstiel, and San Felice.

It will be remembered that in 1897 Dubard, Bataillon and Terre found acid-fast bacilli in large tumors occurring in carp of the fishery at Velars-sur-Ouche (Côte-d'Or). These bacilli can be cultivated at low temperatures and they are pathogenic, producing tuberculosis when inoculated into cold-blooded animals (fishes, frogs, tortoises, lizards, slow-worms, adders).

These bacilli, at first harmless for guinea pigs, rabbits and birds, were

used for successive passage through warm-blooded animals, and it was not long before it was observed that they were producing abscesses with lymph-node enlargements and visceral tuberculous lesions. They appeared, therefore, to have acquired by such passage the essential properties of mammalian tubercle bacilli.

But it was soon discovered that the carp at Velars-sur-Ouche were living in a stream into which the sputum and dejecta of a phthisical subject had been thrown for several months. The fish could therefore have been infected at the same time by both paratubercle and human tubercle bacilli, and the cultures without doubt contained both varieties. On inoculation into mammals the second form developed, and soon predominated, and it was to this alone that the tuberculous lesions produced in the latter were due.

The experiences of W. Kolle, H. Schlossberger and W. Pfannenstiel were published in 1921 (2). From eight different sources they obtained paratubercle bacilli which were used for inoculation into guinea pigs. Passage through successive animals was carried out, and at first the only result was a little nodule at the site of injection. Gradually the cultures became more virulent, the bacilli were found in various organs, and definite tuberculous lesions with lymph-node involvement were produced from which bacilli could be isolated which were of the human type and which only grew slowly at the temperature of 38°.

With the bacillus isolated by Friedmann in 1903 from a tortoise at the aquarium in Berlin tuberculogenic properties were obtained after the tenth passage.

Igersheimer and Schlossberger (3) inoculated paratubercle bacilli into the anterior chamber of the eye of guinea pigs, and stated that serious lesions were produced which resembled those obtained with tubercle bacilli, when they employed paratubercle bacilli that had had several passages through guinea pigs. On the other hand the lesions were only produced with difficulty, and they rapidly cleared up when they used paratubercle bacilli which had not been "passed" through animals.

San Felice (4) states that he has obtained typical tuberculous lesions in the guinea pig with an acid-fast streptothrix isolated from a rat.

Such is an account of the work favorable to the theory that it is possible to transform paratubercle into true tubercle bacilli.

On the contrary, many other workers have completely failed in their attempts to produce this change. Among these we would mention Bruno Lange, who has only obtained negative results in a large number

of well-conducted experiments with paratubercle bacilli from the soil and from water, with bacilli from trumpets, with the acid-fast bacilli of cold-blooded animals, and also with bacilli from the same sources as those used in the experiments of Kolle, Schlossberger and Pfannenstiel. Bruno Heymann and Walter Strauss (5) were also unsuccessful with similar cultures from the Frankfort Institute.

It therefore appears very probable that the experiments of Kolle, Schlossberger and Pfannenstiel, without doubt those also of Igersheimer and Schlossberger, and perhaps also those of San Felice, have been vitiated by some accidental contamination with human tubercle bacilli. It is, moreover, very difficult to exclude such contamination in laboratories where tuberculosis is being studied.

With the collaboration of A. Boquet and S. Nègre, I have taken up the study of this question, using only Moeller's timothy-grass bacillus, which I had already used with C. Guérin in 1905 in our experiments on goats (6). We have carried out subcutaneous, intraperitoneal and intravenous inoculations in a large number of animals, guinea pigs and rabbits. We succeeded in obtaining up to ten successive passages from animal to animal with only one culture in between. The bacilli were isolated from the spleen, each animal receiving 20 to 30 gm. of the microörganisms. We have attempted to produce reinfection, both by means of cultures and by using the organs of the animals used for passage. In none of our experiments have we been able to detect any increase in virulence or any indication that the timothy-grass bacillus could give rise to tubercles. But we found that after several passages through animals the timothy-grass bacillus grew more rapidly at the temperature of 37°, that they produced Koch's phenomenon in tuberculous guinea pigs, and that they did not, however, give rise to any protection against subsequent inoculations of bovine tubercle bacilli.

Consequently, *we have no evidence at least up to the present which would lead us to believe that the paratubercle bacilli can become tuberculogenic by culture in vivo or by passage through warm-blooded animals.*

3. ATTEMPTS AT TRANSFORMING THE TUBERCLE BACILLI INTO NON-TUBERCULOGENIC ACID-FAST BACILLI

Numerous investigators, in particular Ramond and Ravaut, Lubarsch and Mayer, Auché and Hobbs, Ledoux-Lebard, Moeller, Weber and Taute, Von Betegh, Dieudonné, etc., have unsuccessfully attempted to

modify the virus of human or bovine tuberculosis so as to render it pathogenic for cold-blooded animals. Certain workers (Dubard, Bataillon and Terre, Sorgo and Suess, Herzog) thought they were successful, but they discovered later that their cultures had been accidentally contaminated with paratubercle bacilli which were everywhere so abundantly present.

The only result which emerges from this work is that tubercle bacilli injected into, or ingested by the frog, the tortoise or fish, can be recovered from the muscles or the abdominal cavity after a long time.

These bacilli do not even appear to lose their virulence,—as observed by Combemale in 1893. They retain both this and their vitality unaltered for seven months or more (Hermann and Morgenroth). Their essential characteristics, including their tuberculogenic properties, are therefore remarkably stable.

However, J. Ferran (of Barcelona) has cultivated Koch's bacilli in broths in which the quantities of glycerine, glucose and peptone were gradually decreased, and he states that he succeeded in changing them into saprophytic microörganisms, which were mobile, ciliated and non-acid-fast. Cultures of these microörganisms very closely resembled those of *Bacillus coli*.

By inoculating this saprophytic microörganism into a series of guinea pigs Ferran succeeded in reëstablishing its acid-fast characters and its property of producing tuberculosis. It is, nevertheless, certain that this saprophytic microörganism of Ferran does not produce tuberculin, and it cannot be used as an antigen in estimating tuberculous antibodies in fixation reactions.

Auclair and, more recently, Vaudremer (7) have obtained similar results. By cultivating tubercle bacilli deeply immersed in potato broth without glycerine, Vaudremer obtained branching forms which were similar to the leguminous bacteroids, being gram-positive and very easily stained. Transferred to glycerinated potato, these microörganisms again presented the appearance and virulent properties of tubercle bacilli in eight days.

For our part, in collaboration with C. Guérin, we succeeded in causing tubercle bacilli to lose their tubercle-producing properties by maintaining them in a long series of cultures on a medium (potato) saturated with 5 per cent glycerinated ox-bile.

After 230 successive cultures, carried out over a period of thirteen years, we obtained a bacillus which, when transferred to ordinary

glycerine media, had definite characters always acid-fast, but absolutely avirulent for all species of animals and *incapable of giving rise to tubercles.* This bacillus, nevertheless, contained toxic elements, and from it could be obtained a tuberculin quite as active as that from normal tubercle bacilli.

This *nontuberculogenic* bacillus is used by us for vaccinating young animals against virulent tuberculous infection. It is a true Koch's bacillus but physiologically modified. It cannot be considered as a paratubercle bacillus, since it produces a tuberculin equivalent to that from the most virulent tubercle bacilli, it can be cultivated in the neighborhood of 38°, and on animal inoculation it provokes the formation of antibodies titratable against tubercle bacilli as antigens in the Bordet-Gengou reaction.

4. ATTEMPTS AT USING PARATUBERCLE BACILLI FOR ANTITUBERCULOSIS VACCINATION

The attempt has naturally been made to use paratubercle bacilli for vaccinating against virulent tuberculous infection. Experiments in this direction have been made by F. Klemperer, later by Friedmann, Beck, Fritzsche, and Bruno Lange and also, since 1905, by us with C. Guérin.

Klemperer (8) injected a loop of a culture of an acid-fast microörganism obtained from milk into the peritoneum of guinea pigs, twenty-one times over a period of three and a half months. He believed he had obtained in these animals an appreciable resistance against a trial inoculation of virulent bacilli. He only appears to have obtained slight delays in mortality as compared with controls.

Friedmann (9) stated that, in guinea pigs inoculated with the bacillus isolated by him from a tortoise, he obtained a typical tuberculous focus at the seat of injection and that this progressed toward complete cure. Later, the animals thus prepared, and controls, were inoculated at the same time with equal doses of human tubercle bacilli. The controls succumbed in four to six weeks, with lesions of generalized tuberculosis, while the vaccinated animals, after the appearance of ulcers at the site of injection of the virulent bacilli typical of Koch's phenomenon, showed only insignificant signs of lesions in the internal organs when they were killed at the end of three months.

Experiments to confirm these results carried out by Kolle and Schlossberger (10) with Friedmann's bacillus were absolutely negative. It is

extremely probable, therefore, that the cultures used by Friedmann were mixtures of acid-fast tortoise bacilli and human bacilli.

In the experiments which were made with C. Guérin (11) we found that the repeated ingestion of large doses of timothy-grass bacilli by very young goats gave rise to marked infection of the mesenteric lymph nodes which, moreover, cleared up completely. But these glandular lesions gave no protection against inoculations with virulent bovine tubercle bacilli.

Neither M. Beck (12) nor Fritzsche obtained any success in vaccinating calves and guinea pigs against tuberculosis with acid-fast bacilli isolated from butter, nor with Moeller's bacillus.

Finally, Bruno Lange obtained no better results with paratubercle bacilli from the tortoise, the frog or the adder, no more than from the "trumpet" bacilli which he had particularly studied.

CONCLUSIONS

1. It can be stated that up to the present time there is no experimental fact which justifies the hypotheses that there exist in nature, or that it is possible to produce artificially, saprophytic varieties of Koch's bacillus which can be transformed into virulent tubercle bacilli.

2. Nobody has yet succeeded in conferring upon paratubercle bacilli, of whatever origin, tuberculous properties transmissible through series of cases. The only positive results reported in this connection show obvious errors due to superadded infections.

3. Further, there has been no successful attempt to modify true tubercle bacilli by giving them the essential characters of paratubercle bacilli (cultivation at low temperature, rapid growth on glucose or glycerine media, absence of all tuberculogenic properties). The only result has been to show that it is possible to create a race of tubercle bacilli, by cultural devices, which will no longer produce tubercles in susceptible animals, but which still possess the property of secreting tuberculin and of serving as antigen. These bacilli remain toxic for tuberculous animals and will grow only in the neighborhood of 38° on culture media. They are not, therefore, paratubercle bacilli.

4. The paratubercle bacilli cannot be used for producing immunity against active tuberculosis.

5. It is incontestable that tubercle bacilli (bovine, human, avian) form a group of a definitely specific microörganism. The paratubercle

bacilli possess certain biochemical resemblances which link them to the tubercle bacilli, but the hypothesis that they have a common origin does not appear to have any foundation.

6. Consequently *there is no reason for considering paratubercle bacilli, not definitely pathogenic, as offering any danger to man or to animals susceptible to tuberculosis.* They appear to play no part in the spread of tuberculosis in the world at large.

7. In order, however, to shed further light on this subject researches should be carried out in regions where tuberculosis is not yet widespread, with the object of discovering whether varieties of acid-fast bacilli, similar or analagous to those existing to such a large extent in civilized countries, for centuries infested with tubercle bacilli, can be found in these other regions, in such situations as in the soil, in water, in excreta, in manure, etc., as well as on the skins of the inhabitants.

REFERENCES

(1) LANGE: Deutsche med. Wchnschr., 1920, 1921, 1922; and Ztschr. f. Hyg., 1921, xciii, 43.
(2) KOLLE, SCHLOSSBERGER AND PFANNENSTIEL: Deutsche med. Wchnschr., 1921, 437.
(3) IGERSHEIMER AND SCHLOSSBERGER: Ibid., 526.
(4) SAN FELICE: Ann. d'Ig., 1921, xxxi, 457.
(5) HEYMANN AND STRAUSS: Deutsche med. Wchnschr., July 28, 1922, 999.
(6) CALMETTE AND GUÉRIN: Ann. de l'Inst. Pasteur, 1905, xix, 610.
(7) VAUDREMER: Compt. Rend. Soc. de Biol., 1921, lxxxiv, 259; and lxxxv, 1055.
(8) KLEMPERER: Ztschr. f. klin. Med., 1903, xlviii, 250.
(9) FRIEDMANN: Deutsche med. Wchnschr., 1903, no. 50; and 1904, nos. 5 & 46.
(10) KOLLE AND SCHLOSSBERGER: Deutsche med. Wchnschr., December 16, 1920, 1405.
(11) CALMETTE AND GUÉRIN: Ann. de l'Inst. Pasteur, 1905, xix, 601.
(12) BECK: Tuberc. Arb. a. d. k. Gesundh'amt., 1905, iii, 145.

THE ORIGIN OF THE TUBERCLE BACILLUS[1]

STEPHEN J. MAHER[2]

In an address that I made at the Ninth International Conference on Tuberculosis held in Brussels in 1910, I urged the Inner Council to recommend, as the subject for general discussion at the next International Conference, "The Relation of the Acid-Fast Tubercle Bacillus to Other Forms of Bacterial Life." I insisted that the teachers and the textbooks were not telling the truth when they asserted "that the acid-fast tubercle bacillus is always derived from an acid-fast tubercle bacillus, and that only acid-fast tubercle bacilli can be developed from acid-fast tubercle bacilli."

The result of my appeal and my recital of the experiments done by myself and others in support of my heretical contention, was that the tuberculosis authorities of the world merely smiled indulgently and refused to take the matter seriously. However, it is comforting to see that in tuberculosis research as other matters, the mills of the Gods, though they "grind slow" continue to "grind exceeding fine." For now, fourteen years after the International Conference at Brussels, not only does the subject appear on the program of the International Conference as the first question proposed for our discussion, but—perhaps more important—the discussion is opened by our beloved and world-famous tuberculosis authority, Professor Calmette of the Pasteur Institute of Paris.

Here is the question as it appears on our program: "Do there exist in nature or can there be artificially produced saprophytic varieties of Koch's bacillus which can become virulent tubercle bacilli?"

My answer to this question is a double affirmative, "Yes, these varieties do exist in nature, and yes, these varieties can be artificially produced."

[1] This paper was presented at the Fourth Conference of the International Union against Tuberculosis, held at Lausanne, Switzerland, August 5–7, 1924, as part of a discussion on the biological question, "Do there exist in nature or can there be artificially produced saprophytic varieties of Koch's bacillus which can become virulent tubercle bacilli?" It has also been published in the volume of the proceedings of the Congress, pp. 92–96.

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In support of my first "Yes," I offer the following:

In New Haven one hot summer afternoon, a healthy middle-aged house-painter, while hanging in a narrow swing from the roof of a building, accidentally suffered from a severe pinch of his scrotum in the crotch of his underclothing. Because of his hazardous position in the swinging cradle, he could not at once relieve himself, and he continued to suffer the little pain and the discomfort for an hour, until the five-o'clock whistle blew, when he was lowered to the ground. He bathed the small hurt area in cold water. The next morning he noticed that there was a slight redness at the injured spot. Two days later, to the redness there was added soreness. He went to his doctor for advice. The surgeon told him it was a trifling matter, and prescribed a lead wash. At the end of another week he went again to his physician; now there was a marked induration at the site of his injury as large as an English walnut. The doctor, with sterilizing precautions, withdrew an ounce of thick pus and brought it to me for examination. I found it loaded with short, usually straight, bacilli that were acid-fast but not alcohol-fast. I notified the surgeon that I could find no tubercle bacilli in the pus, but that I did find smegma bacilli.

I had been for years vainly trying to cultivate smegma bacilli. I decided that here was an unusual opportunity. I planted the pus on a great variety of media. In some of the fluid media, and on plain agar, there appeared after a few days slight growths of feeble, non-acid-fast rods, but on most of the media there was no growth. I returned the baskets to the incubator, and again notified the physician of my findings. He replied that the little abscess had quickly disappeared after the aspiration, and that he had carefully examined the patient and found that neither in the lungs nor elsewhere was there any sign of tuberculosis.

Three weeks later, I took from the incubator this basket of tubes, intending to discard them. To my astonishment I found on half a dozen tubes of glycerin-broth-potato growing colonies that looked like growths of human tubercle bacilli. They proved to be not only acid-fast but alcohol-fast, typical human tubercle bacilli. I injected emulsions of them intraperitoneally into guinea pigs, and notified the doctor of my alarming discovery. He laughed at me, and told me his patient was quite well and happy at work every day. Three months afterwards, the first of the guinea pigs died of tuberculosis. I killed the others. They all had generalized tuberculosis.

I injected the tubercle bacilli from these guinea pigs into other guinea pigs. They all developed tuberculosis.

"What about the patient?" you would like to ask. Within six months of the time of the injury to his scrotum, the patient was stricken with a very active form of tuberculosis in both lungs, and, within a few weeks, he died. I realize thoroughly that one swallow doesn't make a summer, but here is a case in which apparently the smegma bacillus, "a harmless saprophytic acid-fast bacillus existing in nature," possessed "the property of becoming the virulent tubercle bacillus," thereby justifying my affirmative answer to the first half of our question.

Now as to my affirmative answer to the second half of to-day's question:

In the course of some studies of a strain of *Bacillus mycoides* I found, by allowing cultures to age in normal salt solution and in solid or semi-solid media, composed of salt water and egg yolk, that about half of the small mycoides spores elongated somewhat, and became acid-fast. This mixture of acid-fast and non-acid-fast short, thickish or ovoidal rods, and coccoids was injected intraperitoneally into seventeen rabbits. Two of the seventeen rabbits killed on the sixteenth day were found to present a picture of moderate abdominal tuberculosis, and in the pus of the small nodules were acid-fast rods of the size and shape and tinctorial reactions of tubercle bacilli. On culture, however, they lost their resemblance to tubercle bacilli, and became again the thickish ovoidal or coccoid rods, half acid-fast and half non-acid-fast. This recovered strain was injected into four other rabbits. One of the four died on the sixteenth day. There was a large cheesy mass in the liver. This cheesy mass contained small rods, granular and acid-fast. Cultures from this cheesy mass again gave only mixed acid-fasts and non-acid-fasts, morphologically identical with the original. None of the animals killed after twenty days contained any nodules or other signs of tuberculosis.

This little experiment contains, of course, no proof that these animals developed classical tuberculosis, but it is corroborative of the general proposition that the tubercle bacillus is only one phase of a bacterial cycle.

At the International Conference held in Philadelphia in 1908, and at the International Congress held in Washington in 1908 and at the British Meeting in 1920, and at the International Conference in Paris in 1920, I exhibited a strain of short acid-fast and alcohol-fast bacilli that I had

developed from the acid-fast spores of *Bacillus subtilis* by subjecting these spores successively to the difficult but slightly stimulating environment of normal salt solution and white of egg and human or sheep blood-serum.

Lange has recently reported that after testing on fifty patients "tuberculins" made from saprophytic acid-fasts, and comparing their reactions with control reactions to old tuberculin on the same patients, he had come to the conclusion that there was no essential difference between the response of the tuberculous patient to intracutaneous injections of tuberculin made from pathogenic tubercle bacilli, and the response to "tuberculin" made from saprophytic acid-fasts. In view of this finding, it occurred to my associate, Dr. Lynch, and myself that it would be interesting and perhaps important to compare the tuberculin reactions produced (1) by genuine old tuberculin, (2) by an analogous substance made from my strain of acid-fast bacilli derived from subtilis spores, and (3) by an emulsion of the killed acid-fast spores and acid-fast amorphous spore matter (symplasm of Lohnis) taken directly from an ageing culture of *Bacillus subtilis* on glycerin-agar. This experiment was carried out by Dr. Lynch at the Laurel Heights State Tuberculosis Sanatorium in Connecticut. Intracutaneous injections of normal salt solution, and of glycerin-broth used as controls, were all negative. In the tables (which, by the way, I will not now take time to read) the acid-fast bacillus which I developed from subtilis will be called by its laboratory name *512* and the killed acid-fast spores and symplasm of subtilis will be called *subtilis*.

In brief, the results were that 43 of the 49 patients reacted to old tuberculin, 29 of the 49 patients reacted to *512*, and 5 of the 49 patients reacted to *subtilis*, 4 of the 49 patients gave no reaction, one case that reacted to *512* did not react to old tuberculin, and the proportion of reactions to *512* was highest in the incipient cases and lowest in the advanced cases. It is only fair to say that the *512* reaction was usually less marked than the old-tuberculin reaction, and that the *subtilis* reaction was less marked than the *512* reaction. In a few of the tuberculous patients evidence of the tuberculin and *512* reactions was visible more than a week after the intracutaneous injections.

I need not, before such an audience as this, direct attention to the fact that the so called saprophytic acid-fast bacilli, the acid-fast timothy bacillus, the various acid-fast butter bacilli, the acid-fast bacilli from manure, and many others, have been known for years to possess, under certain conditions of cultivation, most of the cultural and chemical

and serological characteristics of tubercle bacilli. The essential characteristic of tubercle bacilli—the power to cause tuberculosis in a series of guinea pigs—these saprophytic acid-fasts as found in nature were supposed not to possess, and it was the supposed lack of this power that has declassed them, that has caused them to be neglected in most of the research laboratories of the world. Recently, however, they have been coming into their own, and from various scientific centres come reports of a resumption of interest in them, and in their relation to tubercle bacilli:

1. Because of the facility with which the tubercle bacillus, if forced to vegetate in an unfavorable environment, can be made to lose many of its classic characteristics. Professor Calmette has a "biliated" bovine strain that has forgotten how to form tubercles; Dr. Raw has another that does not produce disease of any kind. More than a dozen experimenters have succeeded in effecting radical changes of the tubercle bacillus. I have induced these changes more than a dozen times myself with human, bovine and avian strains. I have taught them to grow at room temperature. I have taught them to grow without most of their waxy capsules. I have taught them to grow deprived of their power to cause tuberculosis. I have taught them to acquire chromogenic power, so that their cultures grew yellow, or pink, or orange or cerise.

2. Because of Much's granules.

3. Because of the facts reported by Calmette himself, and by Gildersleeve and Schultze, that the acid-fast timothy bacillus injected into the veins of rabbits produced tubercles with giant cells, indistinguishable from those produced by the tubercle bacillus.

4. Because Möller, Korn, Mayer and Lubarsch have reported that, with the timothy and the Korn bacillus that had been put through a series of animals, they were able to cause the development of true tubercles with giant cells in injected guinea pigs.

5. Because in many cases of nontuberculous gangrene of the lung have been found acid-fast bacilli, some of which resembled the smegma bacillus, and some the Rabinowitsch butter bacillus, and these had only a light resistance to acids (Folli, Ophuls, Pappenheim and Fränkel).

6. Because in many cases of nontraumatic and nontuberculous pleurisies, and other serous effusions, bacilli more or less acid-fast have been found that grew *in vitro* like tubercle bacilli, but had very little or no power to produce tubercles in guinea pigs.

7. Because of the disturbing frequency with which tuberculosis in housed milch-cows begins in the udders that have been wallowing in manure infected with saprophytic acid-fasts.

8. Because of the autopsy finding of primary tuberculosis in the urinary and sexual apparatus of infants.

9. Because of the unsatisfactory explanations, hitherto devised, of epidemic tuberculosis, of war tuberculosis, and of the devastation by tuberculosis among families and races that suddenly change from rural to urban conditions of life.

In view of these facts how can any one of understanding answer "No" to the question submitted to us for discussion? The scientist who answers "No" must believe that the tubercle bacilli that are killing the patients in our sanatoria and hospitals in Lausanne, Paris, London and New York are the direct descendants of the tubercle bacilli that flourished in the dawn of history and perhaps killed Tut-Ankhamen. Well, the scientist who believes that, must by the same process of reasoning believe that the smegma bacilli to be found on the bodies of most people alive to-day are the direct descendants of the smegma bacilli that were familiars of Cleopatra, and Charlemagne, and Alfred the Great, and Brian Boru!

OBSERVATIONS CONCERNING THE PECULIAR BEHAVIOR OF CERTAIN TUBERCLE BACILLI[1, 2]

WILLIAM M. STOCKWELL

In 1921 Löhnis (1) published his *Studies on the Life Cycles of the Bacteria*, with a *Review of the Literature 1838 to 1918*. He says (2)

> The fact, that all the different phases in the life history of the bacteria, that is, conjunction, formation and development of regenerative bodies, symplastic stage, and the occurrence of different subcycles with peculiar cell morphology, were to be recorded constantly and regularly in all cases studied by us, made the conclusion inevitable that these apparently new facts must have been observed in the course of many previous investigations, though they usually have not attracted much attention, and hardly any of the bacteriological textbooks furnishes information upon them. The practical aims of most of the bacteriological investigations, together with the widespread adherence to standardized methods and theories, have been distinctly unfavorable to thorough studies along such lines. Nevertheless, at least a few references could be given in our preliminary communications, and it was to be expected that a systematic search of the literature would lead to the rediscovery of more confirmative reports, which so far had not found any acknowledgement, or which have been rejected on account of their being not in agreement with the prevalent theory. This expectation has been fully verified.

In his search of the literature for references to studies made on the life cycle of the tubercle bacillus Löhnis made some interesting discoveries, and he states (3) that "Probably thus far the most complete investigation upon the life cycle of *B. tuberculosis* has been made by Maher (1910–1915)."

Most research workers in tuberculosis are familiar with Maher's theories and findings. When I first became associated with him these theories, while very fascinating, nevertheless seemed to be very radical in view of the teachings of the German School with which I was familiar, but after watching his work I was soon convinced by his results that his

[1] From the laboratory at Cedarcrest Sanatorium, Hartford, Connecticut.

[2] Read at the New England Health Institute, Portland, Maine, May 8, 1925.

theories were not as wild as they at first seemed. His advice at all times was "study the tubercle bacillus and study it with an open mind," and this I have endeavored to do.

This particular work has been carried on over a period of four years, and has been reported on in part at the various meetings of the Connecticut State Tuberculosis Commission and medical associates.

In 1920, upon Dr. Maher's return from Europe, where he had been attending the International Conference against tuberculosis, he presented me with a copy of Calmette's book, *L'infection bacillaire et la tuberculose chez l'homme et chez les animaux*, which had just come from the press. The translation of this book by Soper and Smith appeared in 1923.

In his discussion of attempts at vaccination with toxins, Calmette reports work by himself and Guérin with bile-treated tubercle bacilli (4). These tubercle bacilli were grown for many generations on potato saturated with pure bile glycerinated to five per cent. Calmette stated (5) that after "a certain number of successive replants on this medium, the bacillus acquires quite special physiological characters, the cultures greatly resemble those of the bacilli of glanders in appearance, and their virulence diminishes progressively." He also found (6) "that bacilli of human type develop only with great difficulty and very sluggishly in the presence of ox bile, whereas they develop readily in the presence of human bile. The bovine bacillus grows quickly and abundantly on potato with ox bile, while the culture is very difficult on media with human bile." As this had direct bearing on some work on which I was then engaged, I carried out experiments with the human and bovine bile-potato and my results tallied with those of Calmette, that is, the human and bovine strains grew best on human and bovine bile respectively. I was then led to carry out the experiment of growing these microörganisms on glycerinated bile-potato in an effort to change their morphological characteristics.

I selected for this test a rapidly growing bacillus, culture H-32, which I had received several years previously from Professor Fabyan of Harvard University. This was one of Koch's old original cultures. It was subcultured as rapidly as possible, and at each subculture smears stained by both the Ziehl-Neelsen and the Gram methods were examined. Calmette having stated that they resemble the glanders bacillus, I had expected them to begin to lose their gram-positiveness, but after many generations no difference could be discovered between these microorganisms grown on bile-potato and those on other media.

On April 16, 1921, in examining a smear stained by the Gram method, which had been taken from a culture grown on potato, which had been subcultured from glycerine-potato, which in turn had been subcultured from one of the beef-bile-potato tubes, I discovered a few gram-negative bacilli. They would have been very easily overlooked had I not been searching for them, inasmuch as they took the counterstain very poorly, while the gram-positive microörganisms were very darkly stained.

For many weeks I worked on this problem, endeavoring to isolate these gram-negative microörganisms in pure culture, but did not succeed until, in a hanging drop from a bouillon culture, I discovered that there was some slight motility in some of the members present. It then occured to me to try the U-sand filter tube such as is used in isolating motile microörganisms. Plain bouillon was the media used in the U-tube. In 24 hours the filtered side of the U-tube showed the gram-negative motile bacillus. Cultures were made from the filtered sides on bouillon, agar, glycerine-bouillon and glycerinated agar. All but the plain bouillon showed the same as the filtered side, but this bouillon tube in three days showed, besides the gram-negative microörganisms, a surface growth of typical tubercle bacilli, and the following day the filtered side of the U-tube showed the same surface growth. In some way the acid-fast bacilli had gone through the filter the same as the motile gram-negative bacillus. This has been repeated many times with the same result.

There was always the question to settle whether or not this was a contamination. My assistant suggested that, inasmuch as the human bile had come from a draining gall bladder, there was the possibility that some colon bacilli had survived the sterilization of the tubes and that this was what we were finding. Therefore the bile media tubes were discarded, and a control tube of H-32 which was strongly acid-fast and showed no gram-negative element was selected. Subcultures were made from this on plain agar, thence to bouillon, and alternately from bouillon to agar and agar to bouillon for several generations. This transplanting was made as rapidly as possible, and after fifteen generations gram-negative microörganisms similar to the one described before were again discovered. This experiment was repeated as before with the same results. Control cultures on glycerine-agar did not show these gram-negative bacteria. Control specimens of media were likewise negative.

These same experiments have been duplicated with five other strains of tubercle bacilli, namely, B-69 (bovine type), Saranac (human) 37, Saranac (human) C-60 and two of our own laboratory cultures 725 and 726.

The gram-negative microörganisms are slightly thinner than the gram-positive tubercle bacilli, but if stained with dilute carbol-fuchsin the smear has all the characteristics of the tubercle bacillus. In a Gram stain the gram-positive granules can be seen if carefully stained, best smeared from an agar growth. There was no difference noted in either the cultural or staining characteristics between those derived from the human or bovine strains. The young gram-negative bacilli from the bovine culture were distinctly shorter than those from the human strain but after a few generations this difference was not apparent.

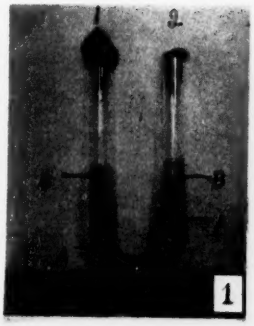
The main points to be observed in isolating these gram-negative microörganism are (1) the absence of glycerine, (2) the media must be neutral or slightly alkaline, (3) the subculture must be made as rapidly as possible, and (4) the U-sand filter tube.

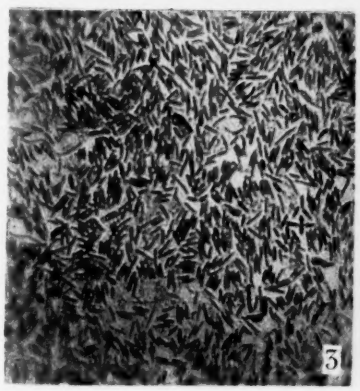
The young gram-negative microörganisms are actively motile; likewise, it has been demonstrated that the acid-fast gram-positive microorganisms may also be motile. When these rapidly growing bacteria showing no gram-negative element are planted on a bouillon U-tube sand filter, it has been observed that within a few days surface growth, resembling in every respect the surface growth from the unfiltered side, will be found on the filtered side. If a hanging drop from the filtered side is observed each day before the surface growth appears motile microörganisms will be found.

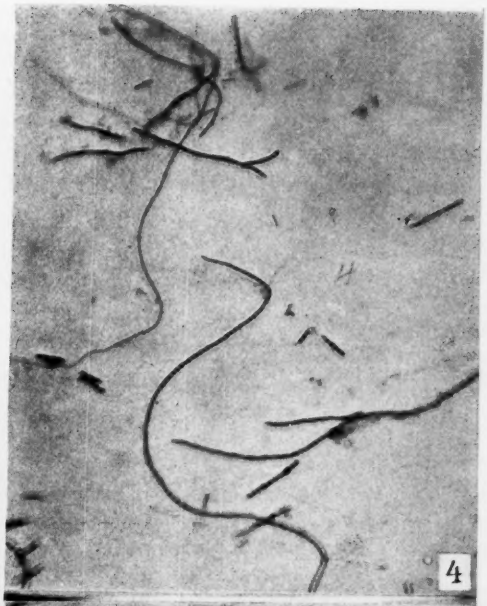
The first thing that I have noticed in these hanging drops is what appears to be a coccus having a violent whirling motion. Within 24 hours tubercle bacilli make their appearance, some of these showng real motility, but upon the appearance of the surface growth these so called whirling cocci and motile microörganisms are no longer found.

The gram-negative bacteria grow in plain and glycerine-bouillon, milk, dextrose broth and on practically all the solid media. However, they do not grow well on any of the media, they being very difficult to subculture and very difficult to keep alive. There is clouding in both glycerine and plain broth, and no pellicle formation. On the solid media growth resembles that of colon bacilli, and there is slight acid production in litmus milk but no coagulation.

We have made many efforts to induce these microörganisms to grow more luxuriantly, and have found that growth, that is, in numbers, is stimulated by the addition of small amounts of certain types of human serum. Out of ten sera only two produced this extra growth. Filtrates







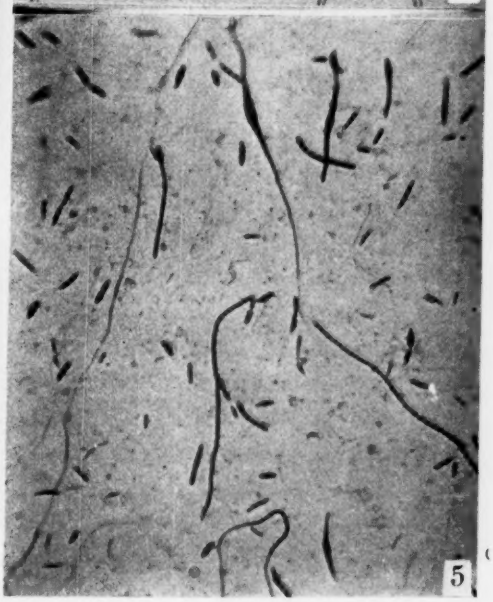


FIG. 1. *A:* Sand level. *B:* Bouillon level. *1:* Inoculated arm. *2:* Filtered arm. The growth of tubercle bacilli may be seen above the level of the bouillon

FIG. 2. A culture four days old on plain agar. Gram stain. × 1200

FIG. 3. A culture six days old on 6 per cent glycerine agar. Gram stain. × 1200

FIG. 4. A growth four days old on 15 per cent glycerine whole egg. Gram stain. × 1200

FIG. 5. A growth four days old on Twort's medium. Gram stain. × 1200

of pus and pleuritic fluid also have stimulated growth, but the reaction of the media seems to be most important.

In two experiments our cultures 725 and 726, both slow-growing and virulent, were planted directly into a neutral broth U-sand filter tube. No effort was made to float the inoculated material on the surface; in fact, the inoculated material all dropped to the bottom of the tubes. These tubes were sealed and placed in the incubator. After six weeks gram-negative microörganisms, similar in all respects to those grown by the rapid subculture method, were discovered. There was no clouding of the broth or surface growth, but a hanging drop disclosed the presence of rather long thin bacilli, a few of which were weakly motile. These bacilli were non-acid-fast and gram-negative, and unless stained and examined very carefully they will be overlooked. While there were a great many of these bacilli in the broth culture it was exceedingly difficult to subculture them, many of the inoculated tubes remaining sterile or at least they did not show any apparent growth. They may be subcultured best in a slightly alkaline, nonglycerinized broth and have a great tendency to die out unless subcultured often.

Probably the most interesting of these observations were from a study of the morphological changes occurring when this gram-negative microorganism was grown on different media. These changes were so great that it was hard to believe that they could have come from one and the same original microörganism. A culture of this gram-negative microorganism, which showed no gram-positiveness, was planted on plain agar, glycerine-agar, whole egg glycerinized to 15 per cent and Twort's medium, and the microphotographs, figures 2 to 5 inclusive, were made from smears on the fourth day stained by the gram method. Figure 2 from plain agar is of bacilli that are entirely gram-negative, and granules, which are both within the bacilli and also free, are undoubtedly regenerative bodies. Figure 3 from glycerine-agar shows the bodies of the bacilli gram-negative but the cocci or regenerative bodies are here gram-positive; they are seen within the bodies of the bacilli, also free; and those free are large and swollen, and many of them are "tack-shaped," the *freie Knospen und Jugendformen* of Meirowsky (7).

Many investigators have described streptothrical forms of tubercle bacilli, and figure 4 shows these forms very well. The arrangement of the regenerative bodies is also well shown; they retain the Gram stain, while the bodies of the microörganisms are gram-negative. It will be

seen that these microörganisms have characteristics of both tubercle bacilli and the colon group. Colonel Nichols (8), while preparing typhoid vaccine at the Army Medical School, has found these same streptothrical forms in his typhoid cultures. On Twort's medium most of the microörganisms are entirely gram-positive; the regenerative bodies are large and swollen; only a few of the microörganisms are gram-negative, and these are thin, and rod-shaped, more like the original plant.

Figure 5 is of the same tube as figure 4, only six days older. Here the microörganisms are broken up, and both gram-positive and negative ones may be seen, the arrangement of the granules being the same in both.

The next step was to endeavor to induce these microörganisms to take on acid-fastness and thereby complete the cycle. As some of those grown on 15 per cent glycerine-egg seemed to hold the Gram stain more intensely and the granule arrangement was so similar to that of acid-fast tubercle bacilli, subcultures were made from them upon 6 per cent glycerine and alternately from this to the 15 per cent glycerine egg. After many generations some of the granules showed acid-fastness and an occasional rod would retain some of the fuchsin. Later, isolated groups (rosettes) of acid-fasts appeared, similar to the young culture on Petroff's medium. These acid-fasts were in all respects similar to the original culture of H-32 that I started with.

That this was a contamination we have practically ruled out for the following reasons: (1) it has never been discovered in control cultures; (2) it has never been discovered in control tubes of media; (3) the difficulty with which it grows and is kept alive; and (4) it gives a positive agglutinating reaction with tuberculous serum.

Ferrán (9) has reported upon his socalled *Alpha bacillus* which, from the short description I can find, appears to be identical with these gram-negative microörganisms that I have isolated. He maintains that this gram-negative is "the tubercle bacillus" and the acid-fast is a mutation.

Being convinced that these microörganisms were one stage in the life cycle of the tubercle bacillus and that, being without their waxy capsule and acid-resisting properties, they might be of value in treating tuberculosis, a series of experiments were carried out. It was found that the gram-negative derived from the fast-growing cultures H-32 and B-69, did not produce tuberculosis in animals. A vaccine was prepared from these microörganisms, and a series of guinea pigs previously inoculated

with tubercle bacilli were treated, but without any good result. They all died, and there was practically no difference in the course of the disease in the treated and untreated guinea pigs.

In another very small series 8 guinea pigs, which had, five months previously, received two injections of this vaccine one week apart, were inoculated by the eye method of Calmette; one died of paralysis but no tuberculous lesions could be discovered. One died of generalized tuberculosis nine months after inoculation, and the lungs showed a fibroid type of disease similar to that seen in the human being. The remaining six were killed one year after inculation. One guinea pig had two tuberculous lesions, one at each pole of the spleen, both of which were fibrotic. The remaining five showed no microscopic lesions of tuberculosis. Nine of the control animals died of acute generalized tuberculosis within three months, while the remaining guinea pig was killed one year after inoculation and no tuberculosis could be found. A larger series cannot be reported on at this time.

REFERENCES

(1) LÖHNIS: Memoirs Nat. Acad. Sciences, Second memoir, 1921, xvi.
(2) LÖHNIS: Ibid., p. 5.
(3) LÖHNIS: Ibid., p. 85.
(4) CALMETTE: Tubercle Bacillus Infection and Tuberculosis in Man and Animals. Translated by Soper and Smith, Williams & Wilkins Co., Baltimore, 1923.
(5) CALMETTE: Ibid., p. 654.
(6) CALMETTE: Ibid., p. 287.
(7) LÖHNIS: Loc. cit., p. 317.
(8) Personal communications.
(9) FERRÁN: Compt. Rend. Acad. Sci., Paris, cxxv, 515.

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No effort was made to float the inoculated material on the surface; in fact, the inoculated material all dropped to the bottom of the tubes. These tubes were sealed and placed in the incubator. After six weeks gram-negative microörganisms, similar in all respects to those grown by the rapid subculture method, were discovered. There was no clouding of the broth or surface growth, but a hanging drop disclosed the presence of rather long thin bacilli, a few of which were weakly motile. These bacilli were non-acid-fast and gram-negative, and unless stained and examined very carefully they will be overlooked. While there were a great many of these bacilli in the broth culture it was exceedingly difficult to subculture them, many of the inoculated tubes remaining sterile or at least they did not show any apparent growth. They may be subcultured best in a slightly alkaline, nonglycerinized broth and have a great tendency to die out unless subcultured often.

Probably the most interesting of these observations were from a study of the morphological changes occurring when this gram-negative microorganism was grown on different media. These changes were so great that it was hard to believe that they could have come from one and the same original microörganism. A culture of this gram-negative microorganism, which showed no gram-positiveness, was planted on plain agar, glycerine-agar, whole egg glycerinized to 15 per cent and Twort's medium, and the microphotographs, figures 2 to 5 inclusive, were made from smears on the fourth day stained by the gram method. Figure 2 from plain agar is of bacilli that are entirely gram-negative, and granules, which are both within the bacilli and also free, are undoubtedly regenerative bodies. Figure 3 from glycerine-agar shows the bodies of the bacilli gram-negative but the cocci or regenerative bodies are here gram-positive; they are seen within the bodies of the bacilli, also free; and those free are large and swollen, and many of them are "tack-shaped," the *freie Knospen und Jugendformen* of Meirowsky (7).

Many investigators have described streptothrical forms of tubercle bacilli, and figure 4 shows these forms very well. The arrangement of the regenerative bodies is also well shown; they retain the Gram stain, while the bodies of the microörganisms are gram-negative. It will be

microörganisms are entirely gram-positive; the regenerative bodies are large and swollen; only a few of the microörganisms are gram-negative, and these are thin, and rod-shaped, more like the original plant.

Figure 5 is of the same tube as figure 4, only six days older. Here the microörganisms are broken up, and both gram-positive and negative ones may be seen, the arrangement of the granules being the same in both.

The next step was to endeavor to induce these microörganisms to take on acid-fastness and thereby complete the cycle. As some of those grown on 15 per cent glycerine-egg seemed to hold the Gram stain more intensely and the granule arrangement was so similar to that of acid-fast tubercle bacilli, subcultures were made from them upon 6 per cent glycerine and alternately from this to the 15 per cent glycerine egg. After many generations some of the granules showed acid-fastness and an occasional rod would retain some of the fuchsin. Later, isolated groups (rosettes) of acid-fasts appeared, similar to the young culture on Petroff's medium. These acid-fasts were in all respects similar to the original culture of H-32 that I started with.

That this was a contamination we have practically ruled out for the following reasons: (1) it has never been discovered in control cultures; (2) it has never been discovered in control tubes of media; (3) the difficulty with which it grows and is kept alive; and (4) it gives a positive agglutinating reaction with tuberculous serum.

Ferrán (9) has reported upon his socalled *Alpha bacillus* which, from the short description I can find, appears to be identical with these gram-negative microörganisms that I have isolated. He maintains that this gram-negative is "the tubercle bacillus" and the acid-fast is a mutation.

Being convinced that these microörganisms were one stage in the life cycle of the tubercle bacillus and that, being without their waxy capsule and acid-resisting properties, they might be of value in treating tuberculosis, a series of experiments were carried out. It was found that the gram-negative derived from the fast-growing cultures H-32 and B-69, did not produce tuberculosis in animals. A vaccine was prepared from these microörganisms, and a series of guinea pigs previously inoculated

with tubercle bacilli were treated, but without any good result. They all died, and there was practically no difference in the course of the disease in the treated and untreated guinea pigs.

In another very small series 8 guinea pigs, which had, five months previously, received two injections of this vaccine one week apart, were inoculated by the eye method of Calmette; one died of paralysis but no tuberculous lesions could be discovered. One died of generalized tuberculosis nine months after inoculation, and the lungs showed a fibroid type of disease similar to that seen in the human being. The remaining six were killed one year after inculation. One guinea pig had two tuberculous lesions, one at each pole of the spleen, both of which were fibrotic. The remaining five showed no microscopic lesions of tuberculosis. Nine of the control animals died of acute generalized tuberculosis within three months, while the remaining guinea pig was killed one year after inoculation and no tuberculosis could be found. A larger series cannot be reported on at this time.

REFERENCES

(1) LÖHNIS: Memoirs Nat. Acad. Sciences, Second memoir, 1921, xvi.
(2) LÖHNIS: Ibid., p. 5.
(3) LÖHNIS: Ibid., p. 85.
(4) CALMETTE: Tubercle Bacillus Infection and Tuberculosis in Man and Animals. Translated by Soper and Smith, Williams & Wilkins Co., Baltimore, 1923.
(5) CALMETTE: Ibid., p. 654.
(6) CALMETTE: Ibid., p. 287.
(7) LÖHNIS: Loc. cit., p. 317.
(8) Personal communications.
(9) FERRÁN: Compt. Rend. Acad. Sci., Paris, cxxv, 515.

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